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THE CAUSE AND ELIMINATION OF THE TOXICITY OF COTTONSEED MEAL FOR SWINE

by

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I. INTRODUCTION

Cottonseed meal is obtained as a byproduct during the processing of cottonseed for oil. Although commercial installations for removing oil from cottonseed have been designed to take advantage of either solvent extraction methods or pressing methods, most installations in the United States contain pressure-exerting machines. Preparation of the seed prior to heat treating and pressing involves cleaning, removal of lint remaining on the seed from the ginning process, and freeing the hulls from the kernels. The kernels, or "meats" as they are called, are then rolled into flakes. Cottonseed cake, a residue of low oil content, results when a major part of the oil is pressed from the heated flakes.

One of two types of presses, either the hydraulic or the expeller, is commonly utilized in cottonseed processing units found in the United States. When the hydraulic type press is used, the meats are cooked in large steam-jacketed kettles previous to expulsion of the oil. The cookers are equipped with mechanical stirrers and provision is made for the addition of water to the meats either before or during the cooking. Only a small percentage of the cookers in the United States are operated under pressure greater than atmospheric, open kettles being in common use. Preparation of the cottonseed meats for

expulsion of the oil by means of the less widely-used expeller type press necessitates only preheating since considerable heat results from the pressure applied.

Cottonseed cake is rich in protein and is utilized as a livestock feed. A portion of the cake produced is broken into smaller pieces and sold as such, and some is ground and reformed into pellets. The largest part of the cake, however, is ground, small amounts of hulls are added, and the mixture sold as cottonseed meal. During the five-year period 1935 through 1939 the average annual production of press cake and meal from cottonseed, flax, peanuts, and soybeans combined was 3,489,000 tons, of which 2,101,000 tons, valued on a 41 per cent protein basis at $53,772,620.00, was produced from cottonseed (141).

Cottonseed meal is usually a cheap source of protein and is fed to all classes of livestock. Its use with poultry and swine, however, as will be shown in the subsequent review of literature, is restricted to certain feeding conditions which limit the amounts that may be fed to these species. The investigation reported in this thesis was planned to study the swine phase of this problem.

II. REVIEW OF LITERATURE

The Use of Cottonseed Meal in the Rations of Farm Animals

As early as 1850, cottonseed meal was reported to exert a deleterious effect on farm animals, which came to be known as "cottonseed-meal injury" or "cottonseed-meal poisoning" and has been the subject of numerous investigations. In a review of early literature Macy (95) points out that this condition has been related to cholin; proportion of cholin and betain; cholin decomposition into neurin, a toxic product; high protein content of the meal; and parasitic organisms in the meal. It has also been attributed to carelessness, ignorance, or lack of cleanliness on the part of the feeder (111); a salt of phosphoric acid (42); ptomaines (53); unsaturated acids (23); excess of acid-forming over base-forming elements (144); deficiency of vitamin B (123); a deficiency of vitamin A (25, 83, 86, 90); and toxicity of a phenol-like substance called gossypol (151, 153).

More recent investigations of the problem have shown that the major factors involved in the cause of cottonseed-meal injury are vitamin-A deficiency and the toxic effect of gossypol. The use of cottonseed meal in the rations of farm animals at present is based on conformity with these findings.

Workstock.

Workstock require mainly energy-producing feeds. One to two pounds of cottonseed meal per head per day is sufficient to furnish the protein needed for balancing the grain rations supplied to these animals. Williams, Jones, and Jones (147) fed these amounts in experiments involving eighty mules and horses, including mares, varying in age from weanling colts to twenty-year olds. No ill effects were observed throughout feeding periods ranging from 224 days to 686 days. These results are supported by those of Mississippi workers (91), who fed as much as three pounds per animal per day to workstock with satisfactory results.

Cattle.

Reports of unfavorable results from feeding cattle on rations containing large amounts of cottonseed meal are common in the early literature. Extensive experiments with cattle by Halverson and Sherwood (83) have shown that typical symptoms of "cottonseed-meal poisoning" can be produced in this species by feeding peanut meal, soybean meal, or linseed meal, with a mineral mixture and poor roughage. Evidence was obtained that failure of cattle on cottonseed-meal rations is due to a deficiency of vitamin A, which may be corrected by good quality roughage. These and similar results by Huffman and Moore (86), Kuhlman, Weaver, and Gallup (90), and Bechdel and Williams (25)

are accepted as conclusive that cottonseed meal is satisfactory for cattle in amounts needed by this species when it is included in a ration that is otherwise adequate.

Sheep.

The results from feeding cottonseed meal to sheep are similar to those for cattle. Although Magee and Darlow in 1920 (98) obtained unsatisfactory gains on lambs fed cottonseed meal with sudan hay and darso silage, Cox in 1929 (41) found low-grade roughage to be the major factor in deaths of lambs being fattened on rations containing cottonseed meal and reported that as much as one pound per lamb per day could be fed with good roughage. The latter work is supported by investigations of Neale (112) who found that 0.8 pound of cottonseed meal per head daily had no ill effects on lambs. Both of these amounts are more than is needed to supply the protein in a grain ration for sheep and, therefore, cottonseed meal is considered satisfactory for this purpose.

Chickens.

Cottonseed meal is successfully fed in combination with an animal source of protein as part of the ration of chickens except in the case of laying hens whose eggs may be placed in storage. According to Sherwood (132), eggs produced from a ration containing cottonseed meal develop a discoloration of the yolk during storage that is referred to as "cottonseed-meal

spots." He found that as little as 2 grams of cottonseed meal per hen daily resulted in some discoloration and concluded that this condition was caused by the oil or something closely associated with it. Schaible and Moore (124) later showed that alteration of the yolk color is due to gossypol present in the meal.

Swine.

Early experiments in the United States with swine rations containing cottonseed or cottonseed meal yielded unfavorable results and many of the experimental animals died. In 1892 Curtis and Carson (44) conducted experiments in which pigs were fed cottonseed meal as well as cottonseed treated by soaking, boiling, and roasting. Some pigs died in each of two trials and, although boiled seed gave the best results, none of the rations was considered safe. Curtiss (43) found that inclusion of cottonseed meal in the rations of hogs increased the rate of gain and lowered the cost, but the use of large amounts proved fatal.

Various means of improving cottonseed-meal rations for hogs were studied by early workers, but a wholly satisfactory method was not obtained. In 1895 Georgeson and coworkers (74) reported that this meal could be used to advantage for short periods of feeding, and later Duggar (51) showed that the feeding of cottonseed meal resulted in fewer fatalities among older hogs than among younger ones. Beneficial results from

supplementing cottonseed-meal rations for hogs with green feed were obtained in 1901 by Burtis (28) and Burtis and Malone (29). Similar results were reported by other workers (32, 47, 48, 55), and in 1932 Godbey (75) fed cottonseed meal free choice with yellow corn to hogs on green forage without ill effects. Iron included in cottonseed-meal rations was found by Withers and Carruth (152) to result in larger quantities of meal being consumed by experimental animals and postponement or averting of deaths. Other workers (27, 81, 120, 143, 146) have reported similar findings but the results have not been sufficiently positive to be widely accepted in practice. Cooking of cottonseed or meal in some of the early experiments gave beneficial results, but complete elimination of the ill effects resulting from these feeds was not achieved. The work of Curtis and Carson (44) with cooked cottonseed has been referred to above. Lloyd of the Mississippi Station (93) fed raw and cooked cottonseed meal and cooked cottonseed in two tests with hogs. Deaths occurred only on the rations containing raw meal, but hogs supplied the cooked feeds refused to eat and became sick. Dinwiddie and Short (49) and Edgerton and Morris (52) reported unfavorable results from cooking treatments. Fermenting of cottonseed meal prior to feeding resulted in little or no improvement in experiments conducted by Dinwiddie and Short (49), Burns (27), and Fuller (54). Limiting the proportion of cottonseed meal fed in hog rations was suggested in 1905 by Fuller (54), who fed ten per cent of the ration in the form of

cottonseed meal without ill effects.

The variable results of the early experiments can be partially attributed to a deficiency of vitamin A which probably characterized many of the rations used before the need for vitamins was understood and which was corrected by pasture with consequent beneficial results. Proof that one or more additional factors are involved, however, was shown by the work of Robison who found (121, 122) that on close observation symptoms of vitamin-A deficiency and cottonseed-meal injury in hogs are different and that the latter can be produced in hogs receiving a balanced ration including cod liver oil, alfalfa, and yellow corn, which are good sources of vitamin A. The injurious effect of cottonseed meal on hogs fed rations adequate in other respects appears to be due to the peculiar susceptibility of this species to gossypol, a toxic substance shown by Withers and Carruth (151) to be present in cottonseed meal. The practical control of gossypol poisoning in the feeding of cottonseed meal to hogs is accomplished by limiting the amounts of meal in the ration to known levels of safety. The amounts recommended are based on the work of Hale (82), who fed cottonseed meal to large numbers of breeding and fattening hogs and determined that this feed could be safely used in amounts up to 9 per cent of the ration or as half of a vegetable-animal protein supplement. This and other work of a similar nature (76, 100, 121, 137) has shown that a combination of vegetable and animal protein constitutes a more efficient protein

supplement than either vegetable or animal protein alone. The application of these principles to the use of cottonseed meal has found wide approval in experimental and practical feeding of hogs. Possibilities of improving these practices lie in making the use of larger amounts of cottonseed meal feasible and eliminating the deleterious effect that even a small amount of gossypol may have. Cottonseed meal contains insufficient protein to balance corn for hogs when fed at a level of 9 to 10 per cent of the ration and at times animal sources of protein rise to price levels discouraging to their use. In addition animal sources of protein, such as tankage and fish meal, are difficult to obtain in many sections of the South, sales points ranging up to a hundred miles or more from some of the farms. Because of these factors a large number of farmers in the South continue to use a limited amount of cottonseed meal alone as the source of protein for hogs. This is illustrated in a survey of 215 farms in Marion County, Alabama, reported by Lanham and Lagrone in 1942 (92), in which all of the feed supplied to hogs other than grazed crops was found to be cottonseed meal and corn. Since no experiments have been conducted to show the existence of a gossypol toxicity threshold for hogs, the possibility exists that cottonseed meal freed of gossypol may prove economical as an only source of protein or more economical than meal containing gossypol when it is combined with an animal source of protein. These considerations prompted the investigation reported in this thesis which was

designed to study the gossypol content of a number of cottonseed meals and determine means of reducing or eliminating the toxicity of these meals for hogs.

The Relationship of Gossypol to the Toxicity of Cottonseed Meal

Occurrence in the cotton plant.

Gossypol was first extracted from cottonseed oil by Marchlewski (99) and from cottonseed by Withers and Carruth (153). It is produced by the internal glands which occur throughout the cotton plant (63, 138), its development in the seed being particularly rapid after the boll begins to crack (59, 64). The amount of gossypol accumulated in the seed appears to be related to the variety of cotton (46, 133), environmental conditions under which the plant is grown (46, 68), fertilization of the plant (68, 142), and oil content of the seed (40, 67, 127).

Chemistry.

Carruth (31) determined that gossypol forms an insoluble substance with aniline and readily forms salts with alkalies. He isolated three substances similar to gossypol as follows: (1) "B" gossypol formed by heating gossypol in the air to its decomposition point; (2) "C" gossypol resulting from fusing gossypol with alkalies to a fairly high temperature and (3) "D" gossypol formed by cooking cotton seed. Menaul (103) found that

pure gossypol exposed to the air and heated to 100° decomposed into water and a reddish brown substance.

In a series of studies on the chemistry of gossypol, Clark (33, 34, 35, 37, 38, 39) found the empirical formula to be $C_{30}H_{30}O_8$. The anilide was found to be a condensation product resulting from the elimination of two molecules of water from one molecule of gossypol and two molecules of aniline, instead of a dianiline salt as suggested by Carruth (31). Clark stated,

> Of the 8 oxygen atoms in gossypol, two have been shown to be present as carbonyl groups, whereas the remaining 6 have been shown to be present as hydroxyl groups. Two of these hydroxyl groups behave differently from the remaining four, being much more acidic and requiring drastic treatment for the hydrolysis of their acetyl derivative.

After studying the substance called "D" gossypol by Carruth (31) Clark (34) says,

> To explain the mechanism of the transformation of gossypol to D gossypol, it has been suggested that in the cooking and pressing process to which cotton seeds are subjected in the manufacture of cottonseed oil, the gossypol present in the seeds is bound by condensing with free amino groups of the seed proteins, forming substances similar in type to dianiline gossypol.

Additional work by Clark demonstrated the presence of a side chain in the gossypol molecule (37). He was able to produce a less toxic substance, apogossypol, by the treatment of gossypol with sodium hydroxide (35, 38), and he studied the action of

chromic and sulphuric acids upon gossypol and some of its derivatives (39).

Adams and associates have recently studied the chemistry of gossypol and synthesized a number of its degradation products (1-22, 30, 105, 106, 109). They point out that three crystalline forms exist having melting points of 184°, 199°, and 214° and that these can be changed from one to the other (30). The common color is said to be yellow but a red form with a melting point of 184°-185° exists which can be converted to the yellow form with solvents or by grinding in a mortar. After summarizing the properties and reactions of gossypol, these workers (11) postulate a structural formula based on two napthaline nuclei connected directly or through an alkylene chain and corresponding to the chemical name 2-2-bi-1, 6, 7, trihydroxy-3-methyl-5 isopropyl-8-aldehydonapthol. It is pointed out that the aldehyde groups are the most active and that this formula allows the existence of two tautomeric forms. Other workers (115, 116) have noted the occurrence of both yellow and red forms of gossypol in cotton seed. Schmid and Margulies (125) prepared pure gossypol with a melting point of 184°.

From the various studies have arisen the terms "gossypol" to designate the original material in the cotton seed, "free gossypol" which is the same material carried through into the meal, "D gossypol" named by Carruth (31) or "bound gossypol" as suggested by Clark (34) to designate the changed form found in

the meal as a result of the milling process, and "total gossypol" used in analytical studies of meal as a summary value of both the changed and unchanged forms. For clarity the terms "bound gossypol" and "free gossypol" are used in this thesis wherever a distinction between the changed and unchanged forms is involved.

Toxicity.

The poisonous nature of gossypol was first shown by Withers and Carruth (151, 153), who demonstrated it to be toxic to fowls, rabbits, and pigs. Other investigators (33, 128, 129) feeding the purified or semi-purified gossypol have confirmed these results. It has been shown by Schwartze and Alsberg (129) that diets containing known amounts of purified gossypol result in rat growth inversely proportional to the levels of gossypol added to the diet. They concluded that the gossypol toxicity threshold for rats is approximately 0.0675 per cent of the ration. Tolerance levels have not been determined for swine and chickens, although Hunt (87) estimated hogs to be four times as sensitive to cottonseed-meal poisoning as rats.

Experiments on the toxicity of bound gossypol have given conflicting results. In the original work showing that gossypol contained in cottonseed was changed in the milling process to the bound form in the meal, Withers and Carruth (154) considered the changed form to be the source of toxicity in the meal. This

study involved the feeding of "short cooked" and "long cooked" meals. The amounts of the two kinds of gossypol contained in the meals were not determined. Gallup (57) studied the toxic effect of three meals with reference to their bound gossypol content. Raw meals, autoclaved meals, and meals previously extracted with ether were fed to rats, the two latter treatments being applied to eliminate any free gossypol present in the meals. He concluded that bound gossypol was responsible for the toxic effect. In later work, published in 1928, Gallup (62) concluded that the toxicity of cottonseed meal may be due to both free and bound gossypol. He stated, "The determination of D-gossypol by the present chemical methods is not a safe criterion for estimating the toxicity of cottonseed products." The same year Clark (34) found bound gossypol "physiologically inert" and in 1929 (36) concluded that it had no influence on the growth rate of rats.

Physiological effect.

The physiological effect of gossypol has been studied with small animals by feeding as well as by injecting purified gossypol and its salts. Schwartze and Alsberg (129) fed gossypol to cats and rabbits for long periods and observed paralysis, nerve degeneration, shortness of breath, cardiac hypertrophy, oedema of the anogenital region and lungs, and effusion into the serous cavities; blood pressure fell, heart action became irregular, and death followed from cardiac involvement. Similar

results with cats and rabbits were reported by the same authors in another study (24), which included rats. Four milligrams usually proved fatal to the latter animals. Schwartze (126) observed that feeding of gossypol to cats and rabbits resulted in paralysis of voluntary muscle and relaxation of the bladder sphincter. Moore noted (108) partial paralysis of the longitudinal muscle and dilation of the upper portion of the small intestine of rats fed gossypol.

Several workers (60, 104, 107) have found incomplete digestion of the proteins of cottonseed products. In studies on the digestion of proteins in vitro, Jones and Waterman (88) obtained this result which they attributed to the interference of gossypol. Gallup (60) found that autoclaving cottonseed and cottonseed meal decreased the digestibility of the protein contained in these materials, but Gallup and Reder (70) presented evidence showing no impairment of digestion or absorption in rats fed gossypol. Olcott (113) extracted cottonseed to remove the gossypol and found this product a better source of protein than commercial cottonseed meal, even after autoclaving. Menaul (101) found gossypol fatal to rabbits in doses of 0.1 gm. administered by mouth. Injections of 0.5 gm. directly into the blood of these animals caused death in four minutes. He studied the effect of gossypol on the erythrocytes and oxygen-carrying capacity of blood by treating blood with gossypol and also by observing the reaction of fish in water containing various percentages of gossypol (101, 102). He concluded that

gossypol prevents the liberation of free oxygen from oxyhemoglobin and exerts a hemolytic effect on erythrocytes.

Gallup and Reder found (73) that hair loss was a frequent occurrence in rats fed diets containing more than 10 per cent cottonseed meal and Gallup (66) showed that rats were more susceptible to gossypol poisoning when receiving diets low in vitamin A content.

Dogs were fed 25 mgm. of gossypol acetate per kilogram of body weight by Macy and Outhouse (97). Death occurred in 26 days with the development of excess abdominal fluid, congestion of all splanchnic organs and a hemorrhagic condition of the intestines. Macy and Mendel (96) found cottonseed kernels unpalatable and toxic to rabbits, guinea pigs, and pigeons. Clinical symptoms observed were emaciation, loss of appetite, weakness, rough hair, unkempt appearance, disturbance in breathing, coma, and often paralysis. Post-mortem examination revealed dilation of the right side of the heart and usually congestion of the liver, kidneys, and, in many cases, the lungs. Splanchnic congestion was frequent and if the intestines were not hemorrhagic they appeared friable and easily ruptured.

Goldberg and Manyard (77, 78) studied the effect of continuous feeding of cottonseed meal to pigs. Pigs receiving 25 per cent of their ration in the form of cottonseed meal died in eight to twelve weeks. Ante-mortem symptoms were failure to eat; vomiting; rapid, short, shallow breathing; anemia; and weakness of leg muscles. Constant post-mortem lesions were

ascites, hydrothorax and hydropericardium with generalized oedema and congestion of intestines, kidneys, lungs, lymph nodes, coronaries, and cerebral meninges. Other workers have described similar symptoms and lesions in hogs (45, 48, 119), in dogs (145), and in sheep and calves (139). Bohstedt, Bethke, and Edgington (26) reported the occurrence of yellow skin, and internal fat in hogs fed cottonseed meal.

Occurrence in cottonseed meal.

The amount and form of gossypol occurring in cottonseed meal is dependent not only upon the amount of gossypol in the seed, which varies with several factors, but also upon the manufacturing process (153, 154) in which more or less of the gossypol may be changed to the bound form. A comprehensive study of the variation in gossypol content of cottonseed meals was made by Sherwood (130) who analyzed forty meals produced in North Carolina. The free gossypol contents of these meals ranged from 0.007 to 0.228 per cent, and bound gossypol contents from 0.335 to 1.076 per cent. Sherwood stated that in the manufacture of cottonseed meal 75 per cent or more of the gossypol contained in the seed is converted to the bound form and pointed out that only 12.5 per cent of the forty meals examined by him contained sufficient free gossypol to exceed the toxicity threshold for rats as established by Schwartze and Alsberg (128). He called attention to the necessity of obtaining information on the amounts of free and bound gossypol

present in cottonseed meals in connection with experiments concerning the feeding value of these meals. Methods for the determination of gossypol have been improved since Sherwood's study was made (84, 134, 135) and it appears probable that the values for free gossypol obtained by him were low. Considering the above and the fact that rats are much more resistant to cottonseed meal injury than are hogs (87), it seems likely that a large percentage of the North Carolina meals would have been toxic to hogs. These results indicate the need for a study to standardize the value of cottonseed meals on the basis of their toxicity and the relation of the toxicity to the free and bound gossypol contents of the meals.

Methods of Eliminating the Toxicity of Cottonseed Meal

Experiments designed to study methods of eliminating the toxicity of cottonseed meal have been concerned with both chemical and heat treatments of the meal.

In 1912, before gossypol was identified as the toxic material in cottonseed meal, Withers and Ray (155) noted that extraction with gasoline rendered the meal non-toxic. Gallup (69) and Gallup and Reder (70, 71) obtained beneficial results from adding two per cent calcium carbonate and two per cent sodium bicarbonate to a cottonseed diet for rats. Several workers (61, 94, 121, 122, 147, 150, 152) have studied the use of iron salts or iron solutions in counteracting the toxicity

of cottonseed meal. The results have been variable. Recently Robison (122) has been able to feed up to 19.0 per cent of cottonseed meal in the ration for hogs by making the meal into a thick slop with water containing iron sulfate. Each 100 pounds of meal was treated with 3.4 pounds of the iron salt. The function of iron in this respect is not understood, although recent work with chickens (140) indicates that the iron combines with the gossypol and prevents the latter from being absorbed.

In 1918 Withers and Carruth (153, 154) found that cooking processes, such as those used in the manufacture of cottonseed meal, change some of the gossypol contained in the seed to the less toxic bound form found in the meal. Since that time several studies have been concerned with this means of eliminating the toxicity of cottonseed meal. Withers (149) found,

> That 50 per cent water and 1 per cent copperas at 100° C. for 1/2 hour changed most of the D-gossypol, but if the experiment was run in an autoclave at 125° C. just as much D-gossypol was changed in the controls which contained water but no copperas as in the samples containing the copperas.

The chemical data were not given and no animal feeding tests were reported. Autoclaved cottonseed meal was fed to pigs by Dowell and Menaul (50) with favorable results. Weanling pigs consumed this meal until gains of 23 to 33 pounds had been attained and no ill effects were observed. Gossypol analyses

of the meal were not reported. Favorable results were also obtained by Gallup (56) from feeding autoclaved meal to rats and weanling pigs. Feeding of the pigs was discontinued after gains of 9 to 35 pounds had been made. In a later experiment the same author (57) studied the effect of autoclaving on the bound gossypol content of three meals and the growth of rats to which the meals were fed. Both the toxicity of the meals and their bound gossypol contents were reduced. The rat results were somewhat variable and destruction of the bound gossypol was not complete. Gallup (58) reported that autoclaving destroyed gossypol more rapidly in cottonseed soaked in water four hours than in the dry seed, and Hassel found (85) that gossypol could be destroyed in ground cottonseed cake by adding 40 to 50 per cent water to the cake and heating it for two hours under 1.5 to 2 atmospheres pressure. A similar procedure for detoxicating cottonseed meal was given by Menaul (103) consisting of raising the water content of the meal to about 40 per cent and heating it above 100° C., preferably with steam under pressure. The time of heating and pressure as well as gossypol analyses and animal feeding data were not given. Clark later reported (36) that rats fed autoclaved meal prepared by the Menaul process showed growth rates inferior to those obtained with untreated meals. Koehn has recently found (89) that moistened cottonseed meal cooked in shallow pans is less toxic to dogs than raw meal. Goldovskiĭ and coworkers (79, 80) have determined that moisture treatment in the

cooking of cottonseed meats results in rupture of the cells and that increasing the degree of the water and heat treatment intensifies the red color of the cottonseed cake.

III. EXPERIMENTAL

Objectives

The objectives of the experiments reported in this thesis were as follows:

1. To study the range in toxicity of cottonseed meals produced by several mills in Alabama.
2. To study the relation of free and bound gossypol in these meals to their toxicity for rats and chicks.
3. To determine the effect of feeding meals containing various levels of free and bound gossypol to swine.
4. To study the reduction or elimination of the toxic effect through treatments of cottonseed meats and cottonseed meal with moisture and heat.

Method of Procedure

General plan.

In this investigation meals were obtained from several mills and analyzed chemically for their contents of both free and bound gossypol. The relative toxicity of the various meals and the effect of moisture and heat treatments in reducing the toxicity were studied with rats and chicks. The

results of these tests were made the basis of an experiment with hogs. A final series of laboratory and oil-mill studies was conducted to determine more specifically some of the factors involved in the reduction of toxicity of cottonseed meal by treatments with moisture and heat.

Methods.

Detail procedure relating to each test is given with the results of the test. The basic methods followed are presented in this section.

Chemical analyses. The meals studied in this investigation were purchased in 100 pound bags and transferred to covered tin cans for storage. Insect infestation was prevented by placing small jars of carbon bisulphide in the containers at frequent intervals. Each meal was sampled from four different places in the container to form the composites that were analyzed. Total gossypol was determined by the method of Smith and Halverson (135) and free gossypol by the revised method of Smith (134). These methods have been shown by the authors (84) to yield a larger percentage of the gossypol contained in cottonseed meal than the older methods, owing to treatment of the meal with moisture prior to extraction.

In the method for determination of total gossypol a two-gram sample is used. The crude oil is removed from the meal by extraction with petroleum ether followed by precipitation of the gossypol with aniline. The resulting dianiline gossypol is

removed by extraction with 90 per cent alcohol, the extract treated to remove extraneous material and the gossypol compound filtered on to a Gooch crucible for weighing. The factor 0.775 is used to convert dianiline gossypol to gossypol.

The free gossypol content of cottonseed meal is relatively small and 60 gram samples are required for the determination of this substance. The sample is moistened and extracted seventy-two hours with ethyl ether. The extract is treated with ethylene glycol and petroleum ether to eliminate foreign material that might interfere with the test, after which the gossypol is precipitated with aniline. The dianiline gossypol which results is filtered on to a Gooch crucible and weighed. The factor used to convert the dianiline gossypol to gossypol is the same as that used in the total gossypol method.

Animal experiments. In the toxicity tests with animals, cottonseed meal was included in a basal ration fortified with vitamin A, which, as pointed out in the literature review, is a critical factor in the feeding of cottonseed meal. Animal sources of protein were used in limited amounts in the chick rations, but excluded from the other rations to avoid the protective influence this type of feed may exert on the toxic effect of cottonseed meal (121).

The rat tests were conducted with male albino rats that weighed approximately 50 grams each when they arrived at the laboratory.[1] The rats were fed laboratory stock ration

[1]The rats used in all of the experiments were purchased from Sunny Hill Rat and Rabbit Farm, Clinton, Maryland.

ad libitum until the adverse effects of shipping had been overcome. When it was evident they had reached a thrifty growing condition they were divided, according to weight, into groups of four rats each, and one group used to test each meal. The rats in each group were housed together in wire cages with removable screens and pans and the several cages kept on a movable cage truck. The pans were changed daily and the screens replaced twice weekly. The basal ration was as follows:

Peanut meal (41 per cent protein)	70.0%
Yellow corn meal	27.5%
Bone meal	1.5%
Salt	1.0%
Cod liver oil - 0.25 cc per rat daily	

The dry portion of the ration was prepared in batches of 1200 grams each and stored in air tight jars. A reserve supply of cod liver oil was kept in a refrigerator, and the amount fed daily to each group of rats was measured out and stirred into the ration at the feeding period. This procedure was followed to insure a high intake of vitamin A regardless of feed consumption and to avoid the possibility of deterioration of the vitamin A during storage of the ration. The diet and fresh water were supplied daily in jars attached to the side of the cage. The food jars were fitted with rims to prevent loss of food and the amounts of food supplied daily were regulated to all that the rats would consume from one feeding period to the next.

The test rations were prepared by substituting cottonseed meal for the peanut meal in the basal ration. The diets

contained a larger percentage of meal than is normally fed in rations for rats. This amount was used in view of the findings of Gallup (65), Hunt (87) and others (117, 118) that the rat is very resistant to cottonseed-meal poisoning. Chicks, which are more sensitive to cottonseed-meal poisoning than are rats, were considered for these tests, but facilities for handling the number of groups required were not available; and Hunt (87) has shown that a complete diet for rats may contain as much as 84 per cent cottonseed meal. The use of a ration lacking an animal source of protein and including a larger than normal percentage of meal was considered permissible for this experiment in that a relative, rather than an absolute, measure of toxicity was desired.

The chick experiments[1] were conducted with White Leghorn chicks hatched by the Poultry Department of the Alabama Polytechnic Institute and placed on experiment when one day old. They were divided at random into groups of 33 each, and one of the cottonseed meals to be tested was supplied to each of the groups in the following ration:

Corn meal	55%
Cottonseed meal	30%
Dried buttermilk	5%
Alfalfa leaf meal	5%
Bone meal	2%
Oyster shell	1%
Salt	1%
Cod liver oil	1%

[1] The tests with chicks were conducted in cooperation with Professor Dale King of the Poultry Department, Alabama Polytechnic Institute.

This ration was used to simulate a normal chick diet in which most of the animal protein is substituted by cottonseed meal. In the second experiment with chicks the above ration containing cottonseed meal that had been treated with moisture and heat was compared with a ration containing balanced proportions of animal and vegetable protein. The latter ration was the stock diet used by the Poultry Department of the Alabama Polytechnic Institute and was made up as follows:

Yellow corn (ground)	30%
Oats (ground)	16%
Wheat bran	16%
Wheat shorts	16%
Meat scrap	10%
Dried buttermilk	5%
Alfalfa leaf meal	5%
Cod liver oil	1%
Salt	1%

The rations were supplied ad libitum and fresh water was provided daily.

The chicks were brooded in batteries with a central heating system. The temperature was thermostatically controlled at 100° F. for the first week and reduced five degrees each week thereafter until a temperature of 70° F. was reached.

The pigs used in the swine experiment included Berkshires, Duroc Jerseys, Poland Chinas, and crosses of these breeds. Following weaning they were treated with a vermifuge and cholera vaccine after which they were fed a stock ration until recovery from the medical treatments was complete. The stock ration was composed of yellow corn, 9 parts and 60 per cent protein tankage, 1 part, by weight. A mineral mixture composed of equal parts

by weight of bone meal, limestone, and salt was available to the pigs during the recovery period. The pigs recovered and attained a thrifty growing condition at an average weight of approximately 34 pounds at which time they were divided according to weight, sex, and breeding into groups of eight pigs each and placed on experiment. Each group had access to a quarter-acre dry lot and a movable house that opened to the south. The basal concentrate mixture fed to the check group was as follows:

Yellow corn (ground)	70%
Peanut meal (41 per cent protein)	25%
Alfalfa leaf meal	5%

The rations containing cottonseed meal were the same as the basal ration except that cottonseed meal was substituted for the peanut meal. Sufficient meal was used to satisfy the protein requirements of the Morrison Feeding Standard for 30 pound pigs (110) when cottonseed meal is used as the only protein supplement to corn. In practical feeding the proportion of meal should be reduced as the pigs grow. However, some farmers fail to follow this plan and some use rough approximations in determining the amounts of feeds that are fed. For these reasons it appeared desirable to provide a margin of safety by continuing the high level of meal, or 25 per cent of the ration, throughout the experiment.

The concentrate mixture was hand fed twice daily in amounts regulated to all the hogs would consume from one feeding period

to the next. Water was supplied twice daily and a mineral mixture composed of the following ingredients was kept before the hogs at all times:

Bone meal	38.80%
Limestone	38.80%
Salt	19.37%
Iron oxide	2.80%
Copper sulphate (anhydrous)	.20%
Potassium iodide	.03%

Weights were taken on three successive days at the beginning and ending of the experiment and at the end of each weekly period while the experiment was in progress. The experiment was closed when the check group exceeded an average weight of 200 pounds per hog.

Oil-mill and laboratory studies. As a basis for these experiments information was obtained on the manufacturing process followed by the mills in producing the several meals studied in this investigation. The data supplied by the mills included maximum cooking temperature, total cooking time and pressure applied in the oil-expelling process. None of the mills were able to furnish definite information concerning the amount of moisture added to the meats prior to cooking.

In the laboratory tests both cottonseed meal and cottonseed meats were subjected to various degrees of heat, moisture, and pressure and the effect of these treatments on the gossypol content of the meal and meats was measured by chemical analyses. A thermostatically controlled oven was used to simulate the heating conditions in open stack cookers and an

autoclave was used to provide conditions comparable to those of pressure cookers. Samples of 100 grams of meats or meal were weighed into beakers of 800 ml. capacity and treated with the amount of moisture, if any, desired. Heat and pressure treatments were then applied, after which the samples were dried under fans and gossypol analyses made.

Following the laboratory studies, oil-mill equipment was used to prepare meals by processing cotton seed and expelling the oil, as is regularly done in the oil mills, and also by reprocessing commercial cottonseed meal.[1] In both cases a small pilot cooker was used first, followed by a larger cooker of approximately normal size. Both cookers were of the single compartment type and could be left open for cooking at atmospheric pressure or closed for applying steam pressure directly to the meats.

Results

Variation in the toxicity of cottonseed meals in relation to their gossypol contents.

Experiments with rats. To compensate for the influence cottonseed from different sources has upon the gossypol content of cottonseed meal, the meals used in this study were obtained

[1] The oil-mill equipment used was made available by the Tennessee Agricultural Experiment Station, and the meals were prepared in cooperation with Mr. A. H. Morgan of that Station. Aid and advice were also received from Mr. J. O. Tankersley of the Tennessee Valley Authority.

from one or more mills that process seed from each of the six major soil regions in Alabama. An effort was made to secure meals of a similar protein content, but all of the mills were unable to supply the same grade of meal. Sixteen meals were obtained that ranged from 36 to 41 per cent in protein content and varied from bright greenish yellow to dark brown in color. The several meals were fed in the experimental diets beginning when the rats averaged approximately 90 grams in weight and continuing 14 weeks. Since growth had practically ceased at the end of nine weeks, the data are presented only for this period. Detailed results of the test are given in the Appendix (Table 1). A summary of the material is presented, along with the protein and gossypol contents of the various meals, in Table 1 of the text.

The data are arranged in Table 1 according to the relative gains of the various groups of rats. Variation between the protein contents of the several meals appears to have no influence on the relative gains of the different groups of rats even though the same level of meal was used in all the rations. This result could be attributed to the fact that all of the rations contained more than the amount of protein normally required for rat growth. It should be pointed out that the protein content of cottonseed meals is adjusted, in the milling process, by adding cottonseed hulls which contain gossypol (41, 138). The data reveal no apparent relationship between the protein content of each meal and its gossypol

content. The total gossypol content ranged from 0.793 to 1.099 per cent; the bound gossypol content from 0.627 to 1.041 per cent; and the free gossypol content from 0.055 to

Table 1

Variation in the Growth Rate of Rats in Relation to the Gossypol Contents of Sixteen Cottonseed Meals Used in the Diets

Meal number	Mean gain per rat[b]	Mean feed consumption per rat	Protein content	Gossypol content of meal[a]		
				Free	Bound	Total
	grams	grams	per cent	per cent	per cent	per cent
0[c]	156	875	41			
16	122	972	36	0.056	0.778	0.834
15	111	861	41	0.055	0.836	0.891
12	110	811	36	0.060	0.819	0.879
13	102	809	41	0.075	0.828	0.903
7	99	738	36	0.067	0.852	0.919
10	99	828	41	0.081	0.904	0.985
9	96	828	41	0.058	1.041	1.099
6	96	794	41	0.078	0.810	0.888
1	94	896	36	0.072	0.844	0.916
8	85	774	41	0.102	0.780	0.882
2	85	726	41	0.102	0.896	0.998
3	70	710	41	0.116	0.750	0.866
11	68	725	41	0.068	0.933	1.001
5	50	512	41	0.166	0.627	0.793
4	36	801	41	0.127	0.820	0.947
14	10	444	36	0.164	0.758	0.922

[a]Dry-weight basis.

[b]Gain calculated at the end of a 9-week feeding period. Each value is the mean gain of 4 rats except for meal No. 14, in which group two of the rats had died and the value shown is the average for the two remaining rats.

[c]Basal ration containing peanut meal.

0.166 per cent. The mean gain per rat of 156 grams in the check group was 34 grams in excess of the gain made by the highest gaining group fed cottonseed meal, indicating that even this

meal may have exerted some toxic effect. Casual inspection of the data reveals some decrease in feed consumption with decrease in gain and a definite inverse relationship between the amount of gain and free gossypol content of the meal fed, but there is no apparent relationship between amount of gain and the percentage of bound gossypol contained in the various meals. Statistical analysis of the data by the multiple regression method, as illustrated by Snedecor (136), gives a multiple correlation coefficient of 0.9239. This value is highly significant, indicating that a high degree of accuracy may be obtained in estimating gains on the basis of the three factors--feed consumed, free gossypol content of the meals, and bound gossypol content of the meals. The betas with their "t" values are as follows:

Gain and free gossypol	By 1.23 =-1.0151	t By 1.23 =5.2036
Gain and bound gossypol	By 2.13 =-0.3431	t By 2.13 =2.3334
Gain and feed consumption	By 3.12 = 0.0993	t By 3.12 =0.4898

The coefficient, "By 3.12" is not significant indicating that the amount of gain made by each group of rats was not closely associated with the amount of feed consumed independent of the effect of the gossypol. The coefficient, "By 2.13", is significant showing that the bound gossypol influenced the gain of the rats in inverse porportion to the amount of this substance present in the meals. The major influence on the amount of gain made by each group of rats, however, was due to the free gossypol content of the meal fed that group since "By 1.23" is highly significant. The

relative influence on the free and bound gossypol on the gains is indicated by the relative sizes of the betas, the beta of gain and free gossypol being approximately three times that of gain and bound gossypol.

The relationship between the free-gossypol content of the various meals and weekly rat gains, based on the data given in Table 2 of the Appendix, is represented graphically by Figure 1 in the text.

This figure was constructed by grouping the various meals, according to free gossypol content, into classes having a range of 0.02 per cent free gossypol each. The mean weekly gains of the rats fed each meal within a class were averaged to obtain the weekly gains for the class. Curves also are shown for the mean weekly gains of rats that received the most toxic cottonseed meal, the least toxic cottonseed meal and peanut meal. The curves representing the gains of rats fed the several groups of cottonseed meal are, in general, related as the percentages of free gossypol of the several groups are related. This indicates the value of free gossypol determinations in estimating the toxicity of cottonseed meal. Lack of complete uniformity between the biological and chemical results may be attributed to the influence of bound gossypol and experimental error in the biological and chemical tests. A comparison of the curve representing the gain of rats fed peanut meal with those of the rats fed cottonseed meals indicates that all of the cottonseed meals exerted some toxic effect. The percentage of

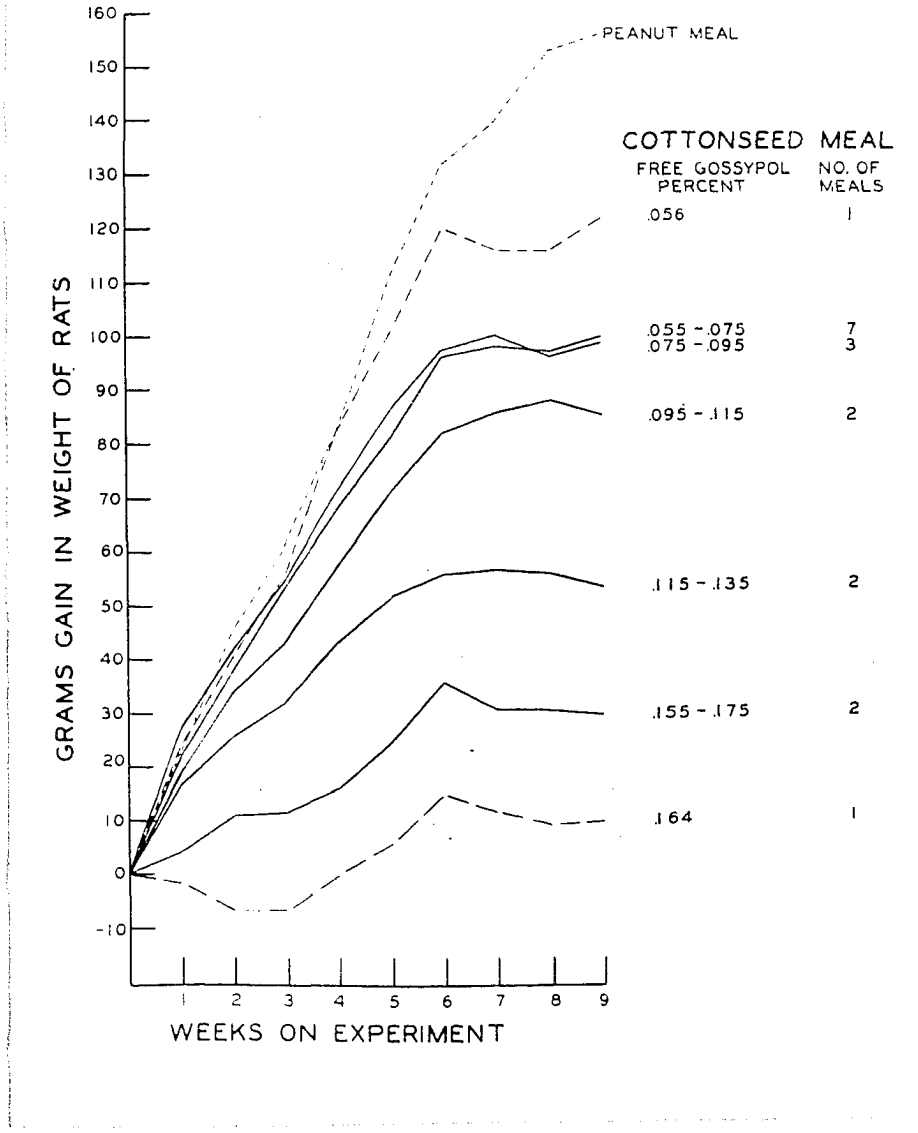

Fig. 1. Relationship of rat gains to the free gossypol content of cottonseed meals included in the diet. The broken lines represent mean weekly gains of a group of four rats fed one meal. The solid lines represent averages of the mean weekly gains of two or more groups fed different meals.

free gossypol in the ration containing the least toxic meal approximates the toxicity threshold for rats that was established by Schwartze and Alsberg (128), with purified gossypol.

As previously indicated, the rats were continued on the experimental diets for 14 weeks. At that time photographs were made of representative rats which had received the least toxic and most toxic cottonseed meals. These are shown in Plates 1 and 2 respectively in the text.

At the end of the experiment the groups of rats which had received the two most toxic and the two least toxic meals were selected for hemoglobin determinations. Blood samples were collected by clipping off the end of the tail, drawing 1/100 ml. of blood into a calibrated pipette and expelling it into a glass cylinder containing a 1 per cent solution of hydrochloric acid. The depth of color was then read with a photoelectric colorimeter which had been previously standardized with a solution of known hemoglobin concentration. Following the collection of blood samples, the rats were killed and post-mortem examinations made. The data resulting from these examinations and the hemoglobin determinations are presented in Table 2 which follows.

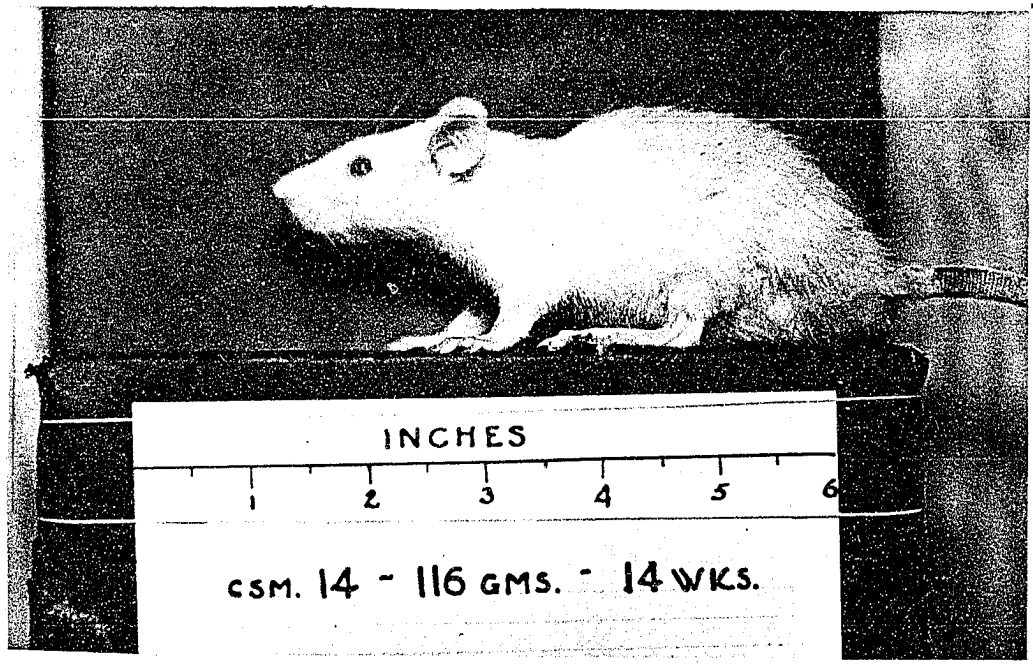

Plate 1. The diet fed this rat included a cottonseed meal containing 0.164 per cent free gossypol.

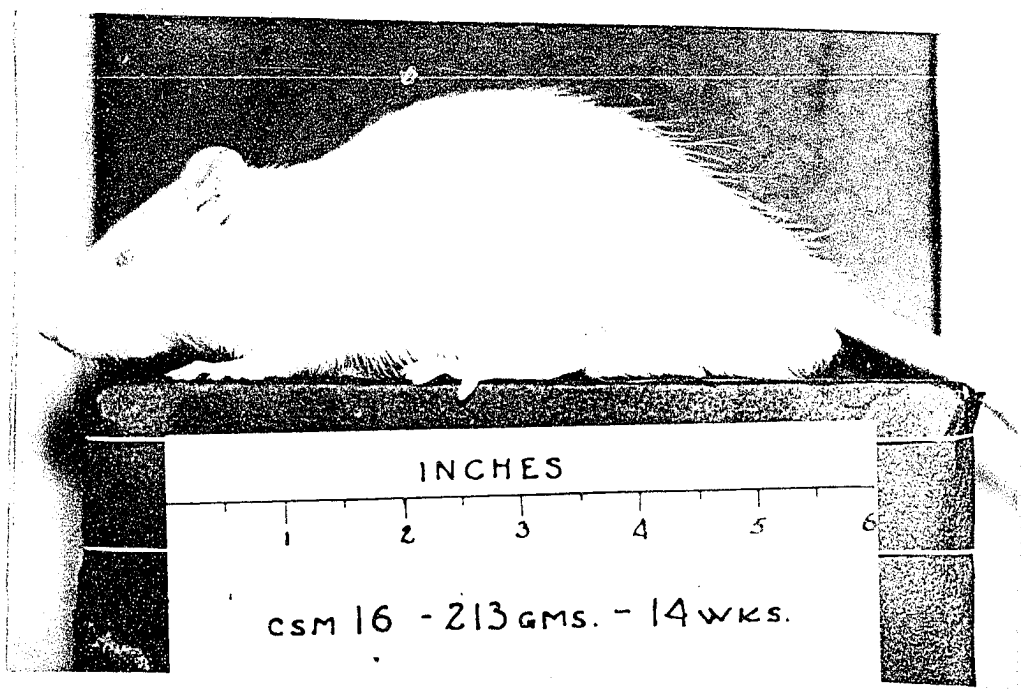

Plate 2. The diet fed this rat included a cottonseed meal containing 0.056 per cent free gossypol.

Table 2

Effect of Kind and Amount of Gossypol in Cottonseed Meals on the Hemoglobin Content of the Blood and Post-Mortem Lesions of Rats to which the Meals Were Fed

Meal no.	Free gossypol content[a]	Bound gossypol content[a]	Rat mark	Hemoglobin content of blood	Gross post-mortem lesions
	per cent	per cent		grams per 100 ml.	
5[b]	0.166	0.627	O	16.5	Congestion and distention middle third small intestine
			R	14.7	Congestion lower two-thirds and distention middle third small intestine
			C	13.4	Congestion and distention middle third small intestine
4[c]	0.127	0.820	O	14.3	Distention middle third of small intestine
			R	14.4	Distention middle third of small intestine
			C	14.6	Distention middle third of small intestine
16	0.056	0.778	O	15.6	Normal
			L	15.5	Normal
			R	15.5	Normal
			C	15.5	Normal
15	0.055	0.836	O	14.8	Normal
			L	18.3	Normal
			R	15.4	Normal
			C	15.6	Normal

[a]Dry-weight basis.

[b]The sample from the fourth rat in this group precipitated before it could be read.

[c]The fourth rat in this group died before the end of the feeding period.

The data are arranged in Table 2 according to the free-gossypol contents of the cottonseed meals that were fed. The mean hemoglobin values per 100 ml. of blood for the groups of rats fed the various meals were as follows: 14.87 grams for meal No. 5 which contained 0.166 per cent free gossypol; 14.43 grams for meal No. 4 which contained 0.127 per cent free gossypol; 15.53 grams for meal No. 16 which contained 0.056 per cent free gossypol; and 16.03 grams for meal No. 15 which contained 0.055 per cent free gossypol. Comparison of the hemoglobin values of the rats that received meal Nos. 4 and 5 with those of the rats that received meal Nos. 15 and 16 indicates a relationship between the free-gossypol content of the meals and hemoglobin values of the blood samples. However, analysis of variance applied to the data gives an "F" value of 1.40 which is not significant, showing that individual variation accounts for the differences. Wide individual variation is also revealed in the range of hemoglobin values from 13.4 grams per cent to 18.3 grams per cent. There is no indication that the bound gossypol contained in the meals influenced the hemoglobin values of the rats to which the meals were fed.

The post-mortem examinations revealed severe damage to the intestines of the rats that received the two meals containing high levels of free gossypol. The viscera of the rats that were fed the meals containing low levels of free gossypol appeared to be normal.

Experiment with chicks. Following the experiment with rats, a ton of meal was purchased from each of the mills that had supplied the more toxic and less toxic samples. Small amounts of the meals were composited from each of the twenty bags in the ton lots and analyzed for free and bound gossypol. Three of the ton lots were selected to represent meals of low, medium, and high free-gossypol content and fed to chicks as outlined in the method of procedure. The results are shown in detail in the Appendix (Table 3) and summarized for discussion in Table 3 which follows.

Table 3

Variation in the Growth and Death Rate of White Leghorn Chicks in Relation to the Gossypol Contents of Cottonseed Meal in the Ration

Meal no.	Total gossypol content	Bound gossypol content	Free gossypol content	Mean gain per chick	Chicks starting experiment	Chick deaths	
						Total	Last 4 weeks
	per cent[b]	per cent[b]	per cent[b]	grams	number	number	number
16C[a]	0.849	0.787	0.062	184	33	10	3
14C	0.805	0.704	0.101	173	33	7	3
5C	0.974	0.722	0.252	79	33	19	13

[a]The letter C designates meal obtained from the same mill that produced the original sample of this number. The second order comprised a ton or more that was composited for both chemical analysis and feeding.

[b]Dry-weight basis.

[c]Feeding period of 6 weeks.

The gains of the chicks, as shown in Table 3, were in inverse order to the free-gossypol content of the meals fed. The detrimental effect of free gossypol is also indicated in the mortality data. Three more chicks died in the group receiving the low free-gossypol meal than in the group receiving the meal of medium free-gossypol content, but the excess deaths occurred during the first few days of the feeding period when the mortality of chicks is usually high. The low gain and high mortality in the group fed the meal containing the largest amount of free gossypol indicate the severe toxicity of this meal. Any influence the bound gossypol may have had is not indicated in the chick gains.

Elimination of toxicity with moisture and heat.

Experiments with rats. In these experiments the effect of heat and moisture on some of the more toxic meals was studied in two series of tests. In the first series two treatments of the original sample of meal No. 14, which had proved very toxic in the earlier experiments, were compared with a mildly-toxic cottonseed meal and with peanut meal. The two treatments applied to meal No. 14 consisted of stirring one batch of meal into cold water and another into boiling water, followed by drying of each. Sufficient water was used to wet thoroughly the meal which was one and one-half times as much water as meal by weight. The meal was stirred into the water to form a wet dough and then dried in a forced-draft oven at

70° C. Twelve hours were required for the drying process after which the meal was ground and mixed into the diets. In the second series of tests a highly-toxic meal, No. 5C, which was obtained from the mill that had produced the original sample of meal No. 5, was given the hot-water treatment and compared in rations for rats with cottonseed meal No. 16 of low toxicity and with peanut meal. Detailed results of the two series of tests are given in the Appendix (Table 4) and summaries are shown in Table 4 of the text.

The results from the first series of tests show that meal No. 14 proved very toxic when fed raw, one of the rats having died and the other three gaining an average of only 16 grams during the 8-weeks feeding period. Both the cold- and hot-water treatments effected marked improvement in this meal, resulting in mean gains per rat of 109 grams and 113 grams respectively. These gains indicate some beneficial effect from the use of boiling water as compared with cold water, but the effectiveness of both treatments was probably influenced greatly by the application of heat during the drying process. The gains made as a result of the water and heat treatments approached that made by the rats which received the mildly toxic meal No. 16, but were considerably below the mean gain of 156 grams made by the rats that received peanut meal. Similar results were obtained in series 2. Meal No. 5C proved even more toxic than meal No. 14 since three of the rats died before the end of the feeding period and the fourth gained only

Table 4

The Effect of Diets Containing Raw and Heat-Treated Cottonseed Meal on the Growth Rate of Rats

SERIES 1	Pea-nut meal	Cottonseed meal			
		No. 16	No. 14	No. 14 heat treated[a]	
				Cold water	Hot water
Free gossypol in raw meal[b], per cent		0.056	0.164		
No. rats per group	4	4	4	4	4
Duration of experiment, weeks	8	8	8	8	8
Mean initial wt. per rat, gms.	78	78	78	78	78
Mean gain per rat, gms.	156	117	16[c]	109	113

SERIES 2	Pea-nut meal	Cottonseed meal		
		No. 16	No. 5C	No. 5C heat treated[a] hot water method
Free gossypol in raw meal[b], per cent		0.056	0.252	
No. rats per group	4	4	4	4
Duration of experiment, weeks	8	8	8	8
Mean initial wt. per rat, gms.	67	66	67	67
Mean gain per rat, gms.	174	138	9[d]	125

[a]These treatments consisted of mixing portions of the meal with one and one-half times their weights of cold or boiling water, as indicated, followed by drying at 70° C.

[b]Dry-weight basis.

[c]One of the rats in this group died before the close of the experiment, and the gain shown is the average of the remaining three rats.

[d]Three of the rats in this group died before the close of the experiment, and the gain shown is that of the remaining rat.

9 grams. Photographs of representative rats fed rations containing the treated and untreated meals in series 2 are shown in plates 3 and 4.

The condition of the rat shown in Plate 3 was typical of the rats fed for long periods on severely toxic meals. The other three rats in this group died. In addition to exhibiting diarrhea and an emaciated condition, these rats lost most of the hair from around the head and neck. The groups of rats fed the wetted and heated meals appeared to be normal in every respect as indicated by the appearance of the rat shown in Plate 4.

To illustrate further the effect of treating cottonseed meal with moisture and heat, a graph was constructed to represent the mean weekly gains of the rats in series 1 whose diets included peanut meal, raw cottonseed meal No. 14, and the latter meal treated with moisture and heat. The data given in Table 5 of the Appendix were used as a basis for the graph which is shown in the text as Figure 2. Examination of this figure reveals that the rats supplied a diet containing the raw meal grew very little while those fed the treated meal made gains approaching that of peanut meal. At the end of eight weeks, however, the rats that received the peanut meal were continuing to gain while those that were fed the treated cottonseed meal had practically ceased to grow.

Experiment with chicks. Following the favorable results obtained with rats from feeding cottonseed meal that had been

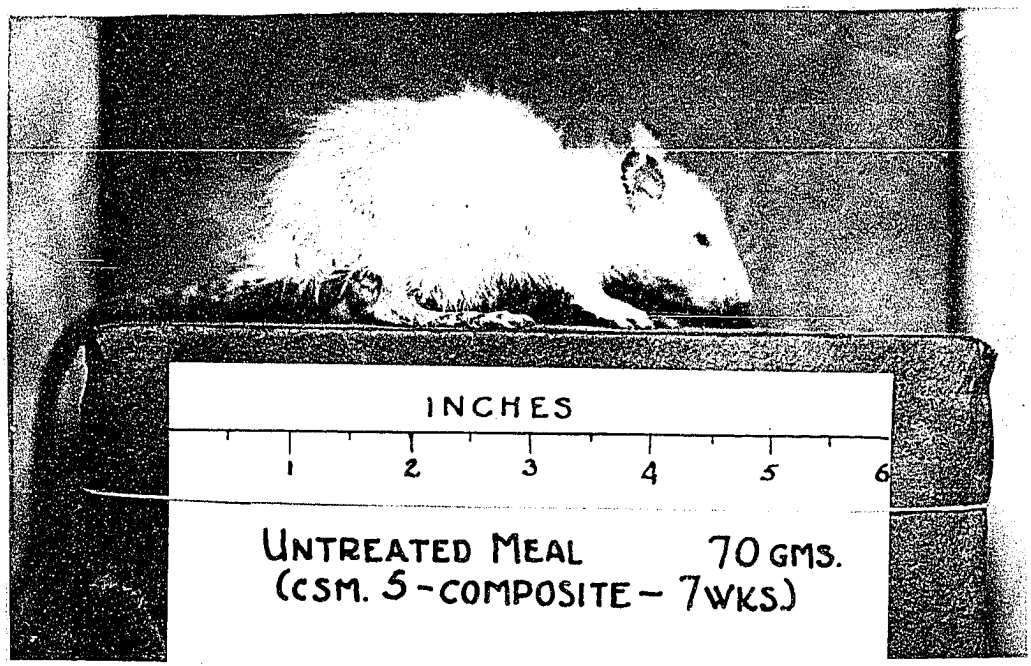

Plate 3. The diet of this rat included a cottonseed meal which contained 0.252 per cent free gossypol.

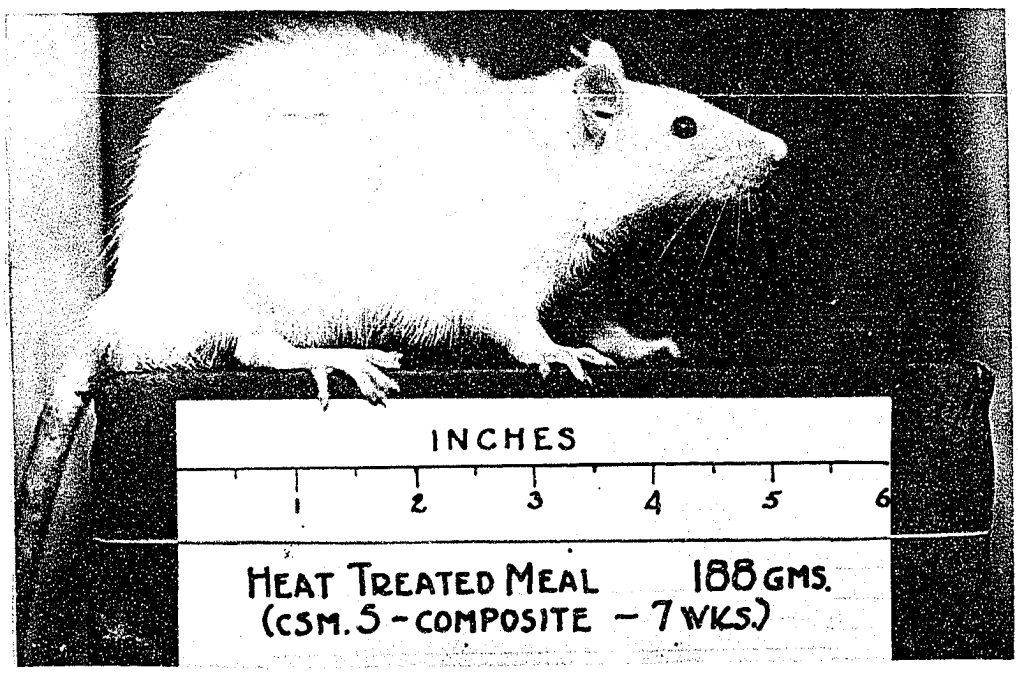

Plate 4. The diet of this rat included the same cottonseed meal as the diet fed the rat shown in Plate 3, except that the meal was treated with moisture and heat prior to feeding.

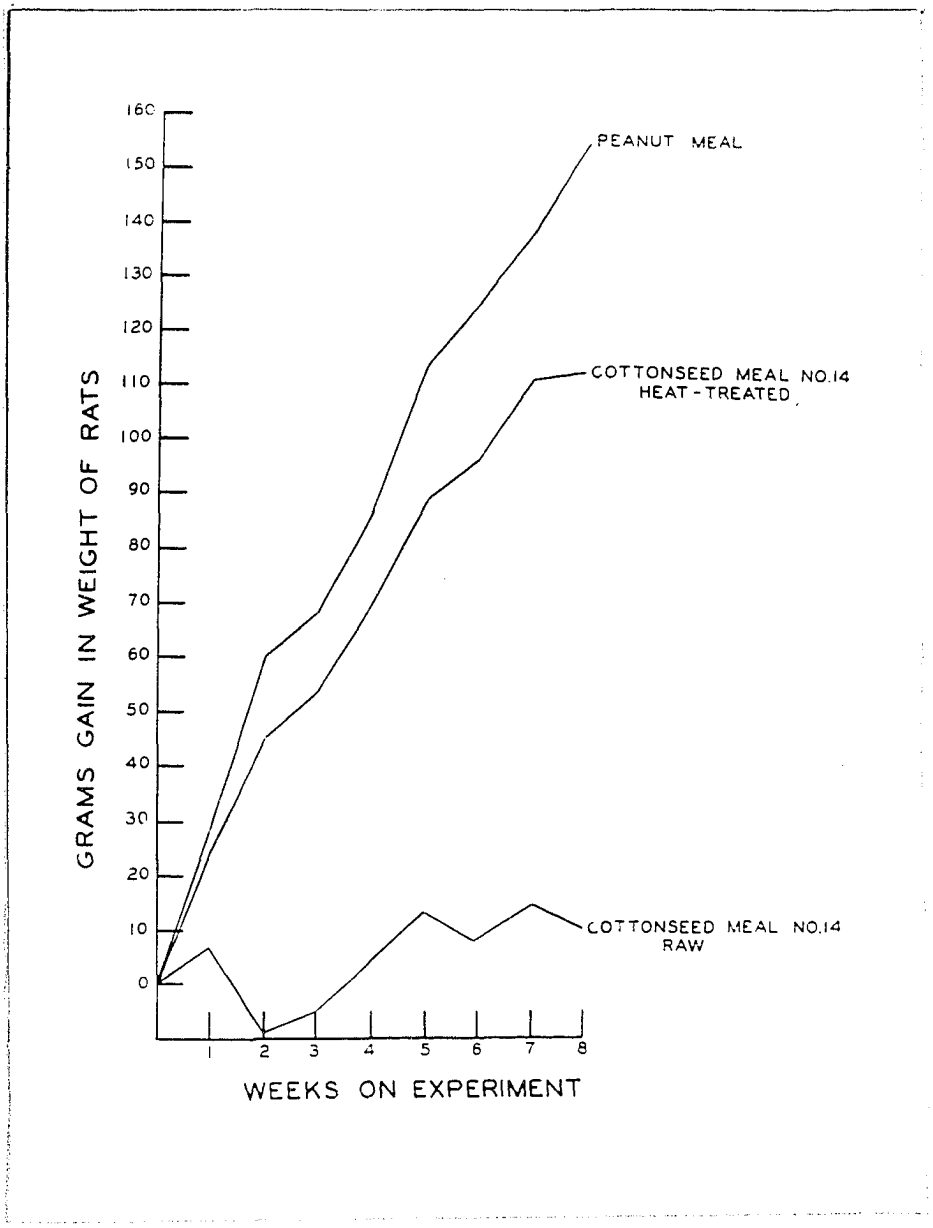

Fig. 2. Response in the growth rate of rats to diets supplemented with raw and heat-treated cottonseed meal and with peanut meal. Each curve represents the mean weekly gain of four rats.

treated with moisture and heat, a test was conducted to determine the response of chicks to meal similarly treated. The treated meal was prepared by mixing a portion of cottonseed meal No. 5C with two and one-half times its weight of water and boiling the mixture for thirty minutes in a steel barrel set over an open fire. The cooked meal was dried in the sun and then incorporated in the basal ration. The raw meal was mixed in the basal ration for one group of chicks and the treated meal was incorporated in the basal ration of a second group. A third or control group was fed the stock ration used by the Poultry Department of the Alabama Polytechnic Institute. The detailed results of these tests are shown in the Appendix (Table 6). A summary of the data is presented in Table 5 of the text.

Inspection of Table 5 reveals that the chicks supplied the stock ration gained an average of 351 grams each during the six-weeks period and none of them died. During the same period the chicks fed the raw cottonseed meal gained an average of 167 grams and eight of them died while those that received the heat-treated or cooked meal gained an average of 224 grams and only three died. At the close of the experiment all the chicks that received the cooked meal possessed a healthy appearance while seven of those that received the raw meal appeared to be near death. From these results it is evident that the stock ration including a wider variety of grains and an animal source of

protein, was superior to the ration in which the cooked cottonseed meal was fed as the main source of protein. However the cooked meal was much more effective in producing growth and livability than was the raw meal.

Table 5

The Effect on the Growth and Mortality of White Leghorn Chicks of Rations Containing Raw Cottonseed Meal and Cottonseed Meal Treated with Moisture and Heat

Supplement	Cottonseed meal 5C raw	Cottonseed meal 5C treated[a]	Control ration[b]
Number of chicks beginning experiment	33	33	33
Mean initial weight per chick, grams[c]	36	36	34
Mean gain per chick, grams	167	224	351
Number deaths	8	3	0

[a]This treatment consisted of mixing the meal with two and one-half times its weight of water and boiling the mixture for 30 minutes in a steel barrel. Heat was applied to the barrel with an open fire. The cooked meal was dried in the sun and then incorporated in the basal ration.

[b]Stock ration used by the Poultry Department of the Alabama Polytechnic Institute.

[c]The experiment was begun when the chicks were one day old and continued six weeks.

The Toxicity of cottonseed meal for swine in relation to the gossypol content and heat treatment of the meal.

When the studies with rats and chicks had demonstrated

that the toxicity of cottonseed meal is due primarily to free gossypol and may be effectively reduced by treatment of the meal with moisture and heat an experiment was conducted to study the significance of these findings in the feeding of cottonseed meal to hogs. Six lots of hogs were fed rations containing the following: (1) peanut meal, (2) cottonseed meal of low free-gossypol content, (3) cottonseed meal of medium free-gossypol content, (4) cottonseed meal of high free-gossypol content, (5) cottonseed meal of high free-gossypol content heat treated in a crock with steam, and (6) cottonseed meal of high free-gossypol content boiled in a steel barrel over an open fire.

Sufficient amounts of the rations containing raw meal were mixed to last approximately two weeks. The treated meals were mixed with the basal ration at each feeding period. The meal heated with steam was prepared daily and that cooked in a barrel was prepared twice weekly. The amounts of total feed needed to supply all that the hogs receiving the treated meals would consume during these periods was estimated and batches of dry meal representing 25 per cent of the total feed were weighed out and cooked. Each of the meals was mixed with two and one-half times its weight of water and the mixtures boiled thirty minutes. The mixture prepared for Lot V was heated in a crock churn by the introduction of steam through a rubber hose and that prepared for Lot VI was heated in a steel barrel, set over an open fire. The prepared meals remained in the

cooking container until just before feeding, when the proportion needed for each feed was approximated and incorporated with the proper amount of basal ration. Detailed results of the experiment are shown in the Appendix (Table 7) and a summary for the accompanying discussion is presented in Table 6 of the text.

Reference to Table 6 shows that a number of hogs died in the course of the experiment. These hogs ate and gained normally until the onset of labored breathing, which was the first symptom of poisoning to appear. The animals exhibited difficulty in obtaining sufficient air by short rapid gasps reflected in movements of the chest and flank, and in several cases by holding the mouth open immediately preceding death, as illustrated in Plate 5.

During the period of labored breathing the hogs were reluctant to move about, and exercise increased the breathing effort markedly. Two of the hogs were observed vomiting. All of the hogs except two died within 12 to 24 hours after the onset of difficult breathing. These two, Nos. 21 and 23 in Lot IV, developed mild symptoms, after which they ate only sparingly and lingered for three weeks before dying. Post-mortem examinations were made on all the hogs. Hydrothorax, ascites, and pulmonary oedema occurred in all cases. The excess fluid in these conditions was straw colored and was present in large amounts in the pleural cavity, as shown in Plate 6.

Table 6

Growth of Hogs Fed Rations Supplemented with Cottonseed Meals Containing Different Kinds and Amounts of Gossypol and Cottonseed Meal Treated with Moisture and Heat

Lot number	I	II	III	IV	V	VI
Supplement	Peanut meal	Cottonseed meal				
		No. 16C Raw[a]	No. 4C Raw[a]	No. 5C Raw[a]	No. 5C treated[b]	
Duration of experiment, wks.	20	20	20	20	20	18[c]
Free-gossypol content, %		0.062	0.107	0.252	0.005	0.003
Bound-gossypol content, %		0.788	0.961	0.722	0.761	
Number of pigs per lot	8	8	8	8	8	8
Mean initial weight, lbs.	33.96	32.92	33.96	34.38	34.17	43.54[c]
Mean final weight, lbs.	201.92	172.04	153.75	105.67	211.42	212.79
Number deaths	0	1	2	6	0	0
Time on experiment prior to death, wks		11	7,17	5,5,6, 9,11,14		
Mean gain per pig, lbs.	167.96	139.13	119.79	71.29	177.25	169.25
Mean daily gain per pig, lbs.	1.19	1.05	0.95	0.90	1.26	1.34
Mean feed consumption per pig, lbs.	730.74	648.86	530.21	285.79	737.41	685.06
Mean feed consumption per cwt. gain, lbs.	435.07	466.39	422.61	400.09	416.03	404.77

[a] The gains and feed consumption of hogs that died were included in the lot totals by using the last weight before death as the final weight and adjusting the feed consumed to the corresponding date on the basis of mean feed consumption per pig per day.

[b] The treatments of meals prepared for both Lots V and VI consisted of mixing the meals in two and one-half times their weights of water and boiling the mixtures for 30 minutes. The mixture for Lot V was heated in a crock churn by the introduction of steam through a rubber hose and that for Lot VI was heated in a steel barrel over an open fire.

[c] Pigs were not available to begin Lot VI until three weeks after the other lots were started at which time pigs with initial weights comparable to those of the other lots could not be obtained.

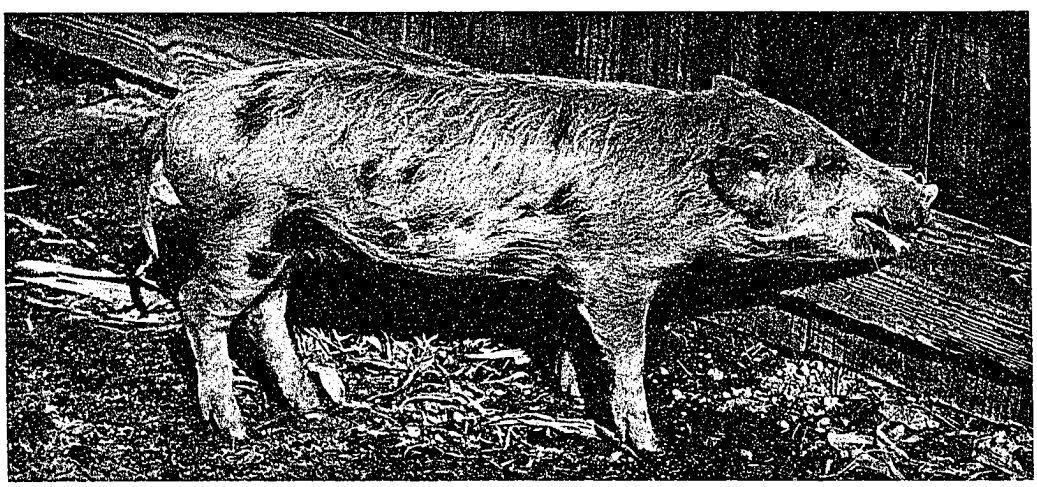

Plate 5. A cottonseed meal containing 0.252 per cent free gossypol was included in the ration received by this pig. The experiment had been in progress five weeks when the pig developed labored breathing and died in a few hours. The photograph was made about ten minutes before death. The straightened condition of the body and open mouth were characteristic of several hogs that died with symptoms of poisoning.

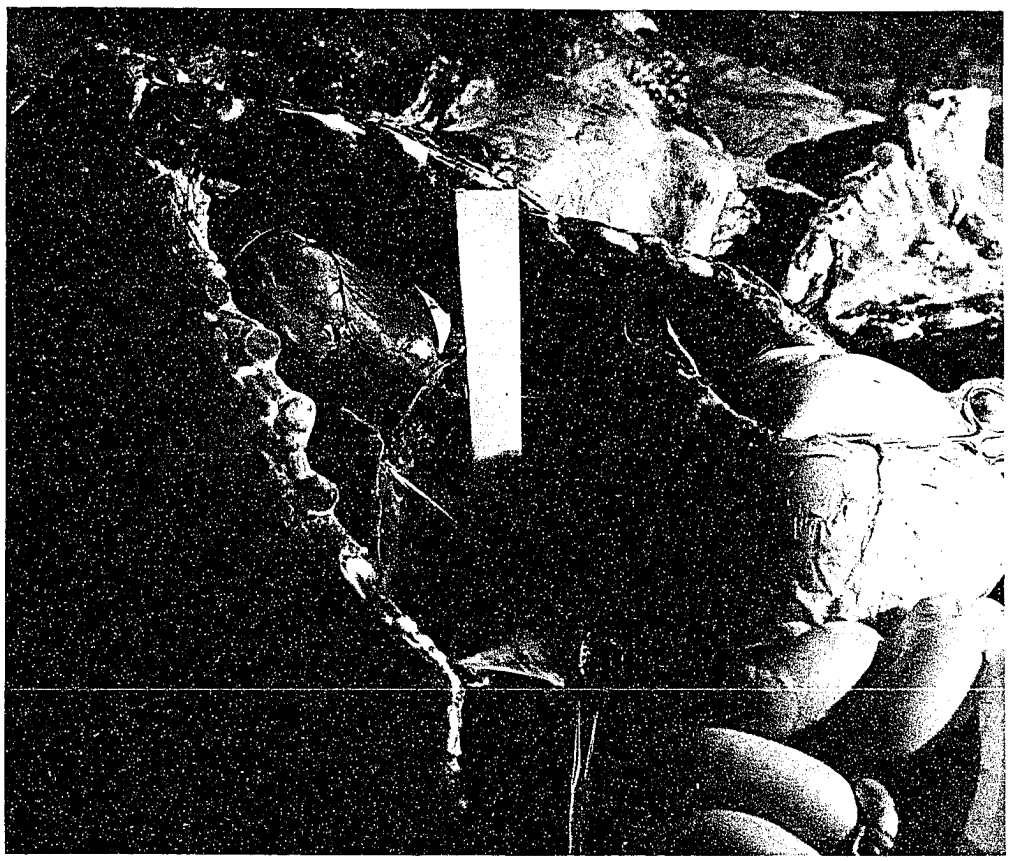

Plate 6. Thoracic and abdominal contents of a pig that died from consuming a ration containing cottonseed meal. The paper strip was placed vertically in the pleural cavity. The cloudy portion of this strip indicates the depth of straw-colored fluid commonly found in the pleural cavities of hogs that die from cottonseed-meal poisoning.

Excess fluid occurred in the pericardial sac of the two hogs, Nos. 21 and 23, whose cases were of a chronic nature, but this condition was not observed in the acute cases. Other conditions observed were oedema of the kidneys, marbling of the mediastinal lymph glands, fibrosis of the liver, hemorrhages in the anterior portion, and injection of the remainder of the duodenum.

The relative influence of free and bound gossypol in causing cottonseed meal to be toxic to hogs is indicated by a comparison of the mortality data from Lots II, III, and IV. The number of deaths that occurred in each lot was closely related to the amount of free gossypol in the meal fed. The meal fed in Lot II containing 0.062 per cent free gossypol resulted in two deaths; and that fed in Lot IV containing 0.252 per cent free gossypol caused six deaths. The number of days elapsing before the initial death occurred in each of these three lots was also in approximate proportion to the free-gossypol content of the meal. The influence of bound gossypol on the mortality rate and gains of these three lots was either nonexistent or so slight that its effect was obscured by that of the free gossypol, since the amount of bound gossypol in the three meals was not greatly different. Lot IV, in which most of the hogs died, was supplied a meal containing the least amount of bound gossypol.

Further evidence that the toxicity of cottonseed meal for hogs is due primarily to the free-gossypol content and that

the toxicity is considerably reduced or eliminated by heat and moisture treatments may be seen in a comparison of the results of Lots V and VI with those of the other lots. The meal fed to Lots V and VI was the same as that fed Lot IV except that it was treated with water and heat previous to feeding. It may be seen that the free-gossypol content of this meal was reduced from 0.252 to 0.005 per cent in the case of the meal treated for Lot V and to 0.003 per cent in that treated for Lot VI. Whereas, six of the hogs in Lot IV died, none of those in Lot V or VI died.

The average daily gain and feed required per unit of gain were more favorable in Lot V, which was fed the steam-heated meal, than in Lot I, which received peanut meal. Statistical analysis of the gains in these two lots gives a mean difference of 9.2 pounds with a standard error of 3.58. The calculated t value of 2.59 is significant. The meal which was prepared by cooking in a barrel over an open fire and fed to Lot VI was included as a practical procedure that could be followed on farms. Pigs were not available to start this group with the others and it was necessary to begin it about three weeks later with pigs of larger initial weight. For these reasons it should be compared with the others in only a general way, but the results are similar to those from the steam-heated cottonseed meal and peanut meal. The condition of all the hogs at the close of the experiment is shown in Plates 7 through 12.

Plate 7. Lot I at the close of the experiment. These hogs were fed a ration supplemented with peanut meal. The average daily gain per hog was 1.19 pounds and the feed required per hundred weight of gain was 435.07 pounds. No deaths occurred in the lot.

Plate 8. Lot II at the close of the experiment. These hogs were fed a ration supplemented with a cottonseed meal containing 0.062 per cent free gossypol and 0.788 per cent bound gossypol. The average daily gain per hog was 1.05 pounds and the feed required per hundred weight of gain was 466.39 pounds. One death occurred in this lot.

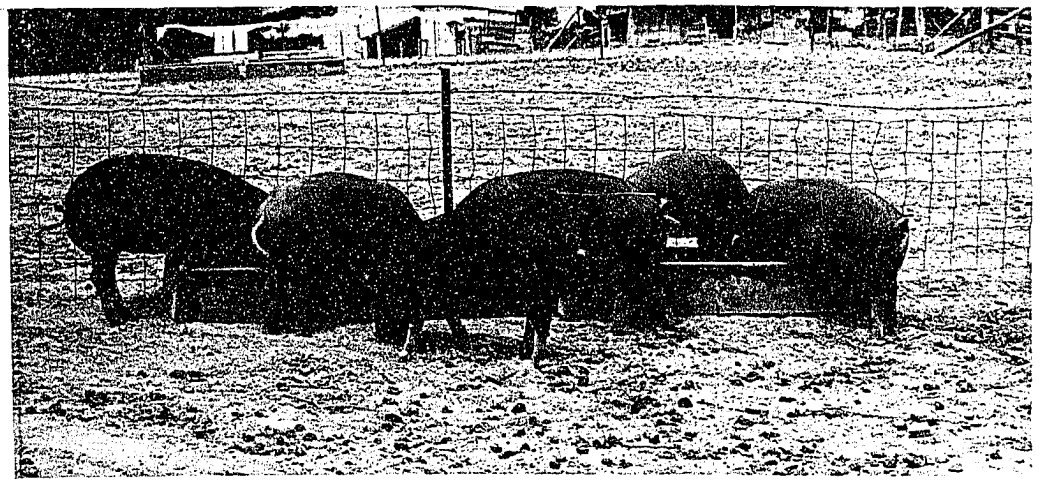

Plate 9. Lot III at the close of the experiment. These hogs were fed a ration supplemented with a cottonseed meal containing 0.107 per cent free gossypol and 0.961 per cent bound gossypol. The average daily gain per hog was 0.95 pound and the feed required per hundredweight of gain was 442.61 pounds. Two deaths occurred in this lot.

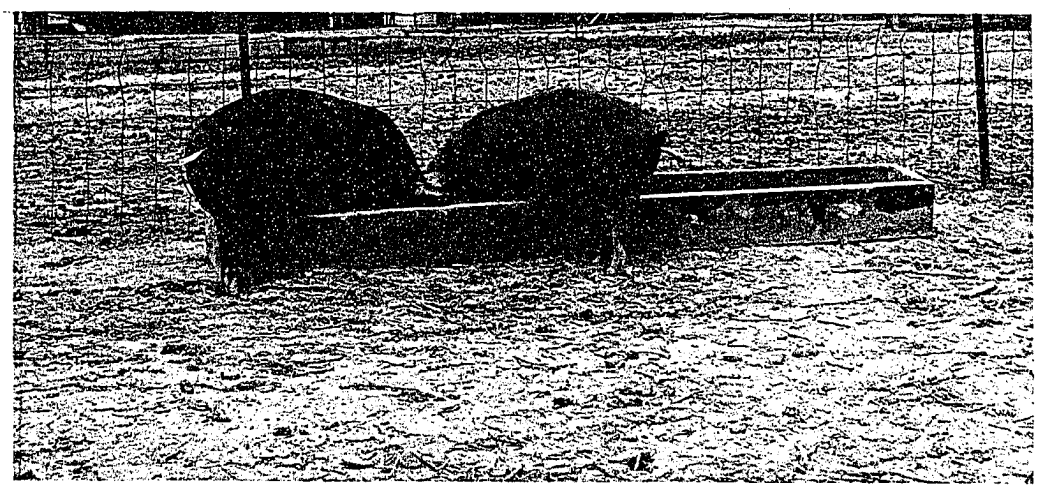

Plate 10. Lot IV at the close of the experiment. These hogs were fed a ration supplemented with a cottonseed meal containing 0.252 per cent free gossypol and a 0.722 per cent bound gossypol. The average daily gain per hog was 0.90 pound and the feed required per hundredweight of gain was 400.09 pounds. Six deaths occurred in this lot.

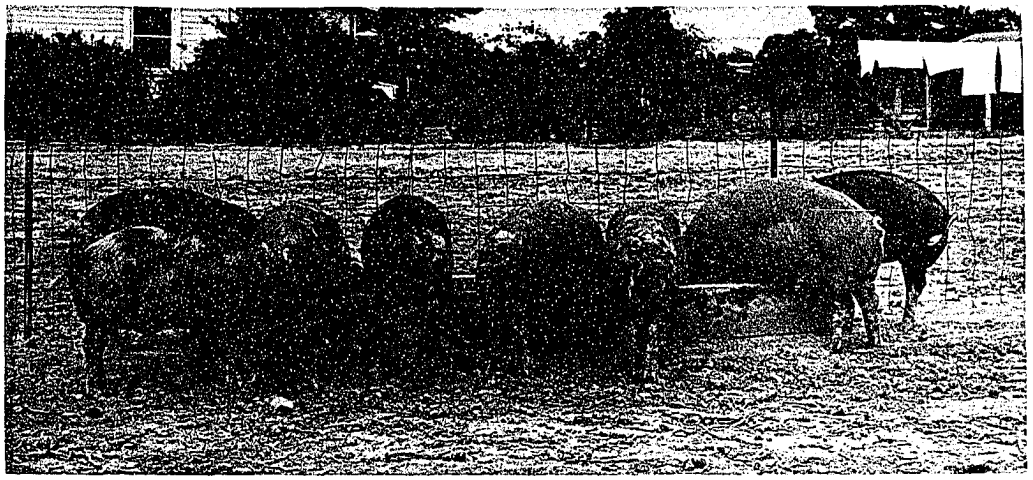

Plate 11. Lot V at the close of the experiment. These hogs were fed the same cottonseed meal as those shown in Plate 10 except that the meal was treated with water and heated with steam prior to feeding. Following treatment, the meal contained 0.005 per cent free gossypol and 0.761 per cent bound gossypol. The average daily gain was 1.26 pounds per hog and the feed required per hundredweight of gain was 416.03 pounds. No deaths occurred in this lot.

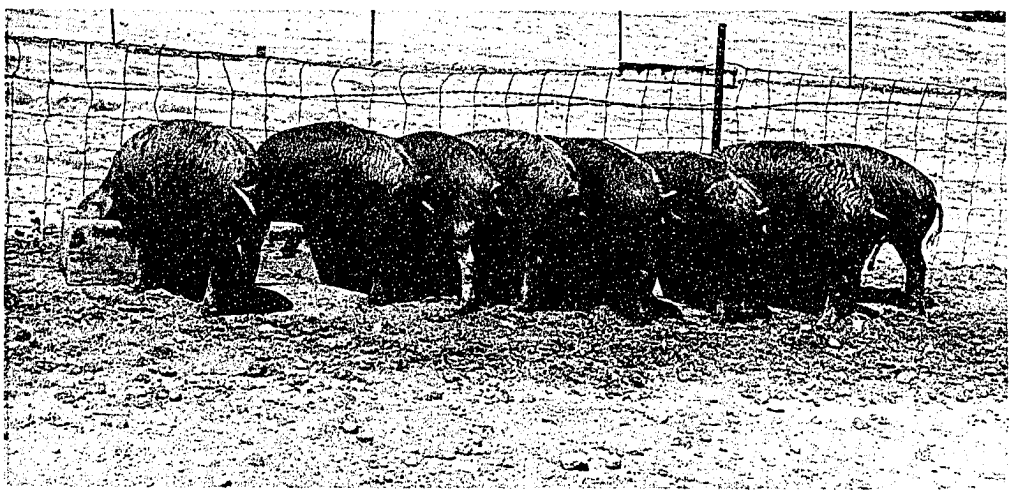

Plate 12. Lot VI at the close of the experiment. These hogs were fed the same cottonseed meal as those shown in Plate 10 except that the meal was treated with water and cooked over an open fire prior to feeding. The average daily gain was 1.34 pounds and the feed required per hundredweight of gain was 404.77 pounds. No deaths occurred in this lot.

Processing factors affecting the kind and amount of gossypol contained in cottonseed meal.

Oil mill practices. Processing data were obtained from the mills that had produced the original 16 samples of meals that were analyzed for free and bound gossypol. All of the mills use stack cookers except one, which uses a single-compartment pre-heating cooker in connection with an expeller press. In general, the mill cookers are operated to raise the temperature of the incoming meats to a point between 212° F.[1] and 240° F. during the last 20 to 30 minutes, the entire process requiring from one to two hours. The degree of heat, time of heating, and amount of moisture used vary not only from mill to mill, but also from time to time in the same mill. In general, the amount of water added to the cottonseed meats prior to cooking is based on the pressing condition and consequent degree of wetness of the meats issuing from the cooker. The latter condition is judged according to the appearance and feel of the cooked meats. In most of the mills the water is let into the meats from an ordinary faucet regulated by hand and as a result definite information regarding the amount of water used could not be obtained. Data concerning the degree of heat, time of heating, and pressure used in the oil-expelling process were obtained

[1]The Fahrenheit scale is commonly used in the mills to express temperature readings and the temperature data concerning mill practices and the use of mill equipment are shown in degrees Fahrenheit.

and are presented in Table 7 along with the gossypol analyses of the corresponding samples of meal.

Table 7

Variation in the Gossypol Contents of Cottonseed Meal with Relation to Oil-Mill-Processing Factors

Meal no.	Free gossypol in meal	Bound gossypol in meal	Maximum cooking temperature	Total cooking time	Pressure used in expelling oil
	per cent[a]	per cent[a]	degrees F.	min.	lbs./ sq. in.
15	0.055	0.836	230	130	4,000
16	0.056	0.778	240	96	4,000
9	0.058	1.041	230	130	4,000
12	0.060	0.819	233	105	4,000
7	0.067	0.852	240	60	4,000
11	0.068	0.933	220	105	not given
1	0.072	0.844	225	120	4,500
13	0.075	0.828	235	90	4,200
6	0.078	0.810	230	72	4,000
10	0.081	0.904	235	90	4,200
2	0.102	0.896	235	60	4,200
8	0.102	0.780	229	96	4,000
3	0.116	0.750	Information not given		
4	0.127	0.820	206	100	4,000
14	0.164	0.758	240	96	4,000
5	0.166	0.627	Estimated to be low	15 (est.)	not given (expeller process)

[a]Dry-weight basis.

In analyzing the data it should be remembered that the practices in each mill are changed from time to time, and that the amount of water used is a source of variation not disclosed by the table. The amount of pressure used in expelling

the oil varies but little from mill to mill. Nine of the mills used 4000 pounds per square inch, and the other five that furnished this information use either 4200 or 4500 pounds per square inch. The data indicate that the pressure applied to the cooked meats in expelling the oil has little or no influence on either the free- or bound-gossypol content of the meal. Examination of the data reveals no definite relationship between either the free- or bound-gossypol content of the meals and the maximum temperature at which the meats are cooked, but there is evidence of decrease in the free-gossypol content of the meals as the cooking time is increased.

Meal No. 5 is particularly interesting in that the process of manufacture is distinctly different from the others. This meal has a bright greenish-yellow color and a high protein content, which by cottonseed-meal standards would be rated the most desirable of all the meals for feeding livestock. As indicated in Table 7, the original sample had a free-gossypol content of 0.166 per cent, the highest of all the samples tested, and additional lots purchased for feeding in the chick and hog experiments contained as much as 0.252 per cent free gossypol. Although exact information relative to the process used in making this meal could not be obtained, the cooker and press were observed in operation. It was estimated that the meats remained in the cooker about fifteen

minutes and the maximum temperature reached was considerably less than that in the other mills.

Laboratory studies. Information obtained from the mill data was used as a basis for laboratory studies to determine more specifically the effect of moisture and heat on the gossypol content of cottonseed meats and meal. Amounts of moisture and temperature ranges that have possibilities of use in present milling practices were studied. The interrelationship of amount of moisture, degree of heat, and duration of the heating period necessitated several series of tests.

The effects of pressure and moisture on gossypol in cottonseed meal are shown in Table 8 in the text. In these tests the temperature was regulated to approximately 105° C. which is intermediate in the range of temperatures employed by the mills. The length of time heat or pressure were applied was based on the milling practice of bringing cottonseed meats to the maximum temperature toward the latter part of the cooking period and holding them at that temperature for twenty to thirty minutes as well as the length of time required to drive all the moisture from the material. As indicated in the table the free-gossypol content of the raw meats and meal was 0.641 per cent and 0.219 per cent respectively.

Series I shows that the reduction of the free-gossypol contents of both the meats and meal was accelerated by increasing the moisture content previous to heating. However, meats

and meal containing 21 per cent moisture retained 0.298 and 0.032 per cent free gossypol respectively after heating at 105° C. for 60 minutes. The moisture was driven off and all

Table 8

Effect of Moisture with and without Pressure on the Free Gossypol Content of Cottonseed Meats and Cottonseed Meal when the Degree of Heat is Held Constant

Series	Sample no.	Moisture content	Temperature of meats & meal[b]	Duration of temperature or pressure	Pressure	Free-gossypol content[a]	
						Meats	Meal
		per cent	degrees C	min.	lbs. per sq. in	per cent	per cent
Raw meats and meal						0.641	0.219
Series I (oven treated)	1	7	105	60	None	0.555	0.059
	2	9	105	60	"	0.544	0.066
	3	13	105	60	"	0.396	0.044
	4	17	105	60	"	0.324	0.058
	5	21	105	60	"	0.298	0.032
Series II (oven treated)	6	30	105	15	"	0.216	0.036
	7	46	105	15	"	0.055	0.014
	8	63	105	15	"	0.013	0.015
Series III (autoclaved)	9	17	105	30	10	0.411	0.058
	10	30	105	30	10	0.200	0.027
	11	46	105	30	10	0.012	0.012
Series IV (autoclaved)	12	46	97	1	5	0.440	0.159
	13	46	97	15	5	0.255	0.100
	14	46	97	30	5	0.137	0.029
	15	46	105	1	10	0.289	0.130
	16	46	105	15	10	0.134	0.048

[a]Dry-weight basis.
[b]Temperature of autoclave in series III and IV

the samples scorched before the cooking period was over, indicating that no further reduction could be obtained by continued

heating without the addition of moisture. In series II moisture levels of 30, 46 and 63 per cent were used. The meats and meal were heated to 105° C. as in series I, but this temperature was held only 15 minutes, which was the time required to drive most of the water from the sample containing 30 per cent moisture. In this series, as in series I, the free-gossypol content of both the meats and meal was reduced in approximate proportion to the amount of moisture contained in the material treated. The free-gossypol content was reduced to 0.014 per cent in the meal containing 46 per cent moisture and to 0.013 per cent in the meats containing 63 per cent moisture.

In series III are shown the results of constant pressure applied in an autoclave to meats and meals containing different amounts of moisture. In this series as in the previous ones the reduction of free-gossypol content was in rough proportion to the moisture. Practically all of the free gossypol was eliminated in both the meats and meal at the moisture level of 46 per cent.

Series IV shows the effect on the free-gossypol content of meats and meal of high moisture content when pressures of 5 and 10 pounds were applied from 1 to 30 minutes. The free gossypol content of both the meats and meal was reduced in approximate porportion to the amount of pressure and length of time it was applied, but in none of the tests in these ranges was the

free gossypol changed as completely as in Series III where 10 pounds of pressure was applied for 30 minutes.

The effects of temperature, time, and method of heating on gossypol in cottonseed meal are shown in Table 9 which follows.

Table 9

Effect of Temperature, Time and Method of Heating on the Free and Bound Gossypol Content of Cottonseed Meal and Meats of High Moisture Content

Sample no.	Material treated	Moisture content	Treatment	Duration of treatment	Gossypol[a] Free	Gossypol[a] Bound
		per cent		min.	per cent	
1	Meal 5C		Raw		0.252	0.722
2	"	53	Oven heated to 50° C.	30	0.116	0.835
3	"	53	Oven heated to 75° C.	60	0.058	0.834
4	"	53	Oven heated to 90° C.	90	0.025	0.787
5	"	53	Oven heated to 97° C.	150	0.021	
6	"	53	Oven heated to 102° C.	180	0.008	0.786
7	"	67	Boiled in churn with steam	15	0.009	
8	"	67	Boiled in churn with steam[b]	30	0.005	0.761
9	Meal 4, 5, 14C		Raw		0.125	
10	"	67	Boiled in barrel, over open fire[b]	1	0.009	
11	Meats		Raw		0.641	
12	"	46	Autoclaved at 10 lbs. pressure	30	0.012	0.423

[a]Dry-weight basis.

[b]After treatment these samples were allowed to cool in the barrel before analyses were made. The cooling process required about five hours.

Meal No. 5C which contained 0.252 per cent free gossypol prior to heating was used in the series of oven treatments designated as samples 2, 3, 4, 5, and 6. These samples were placed in the oven at the same time and one removed at each of the temperature levels shown in the table. The free-gossypol content was reduced over half by the time the temperature of the meal reached 50° C. which required 30 minutes. Reduction continued with the increase in temperature and length of time the heat was applied. However, the rate of reduction became slower, particularly after the level of 0.058 per cent of free gossypol was reached, and at 97° C. after two and a half hours of cooking the meal still contained 0.021 per cent free gossypol. When the temperature was raised to 102° C., which required only 30 minutes additional heating, the free-gossypol content was reduced to a trace. This indicates that the boiling point of water is a critical temperature for destruction or conversion of free gossypol. This is supported by the results of samples 10, 7, and 8, in which the free-gossypol content was roduced to negligible amounts by boiling for 1 minute, 15 minutes, and 30 minutes respectively.

The bound-gossypol content of the raw meal No. 5C was 0.722 per cent. The initial heat treatment of this meal increased its bound-gossypol content, but not enough to account for the loss in free-gossypol content. Continued treatment with heat lowered the bound gossypol slightly but after the temperature had reached 102° C. and the meal had been

subjected to heat three hours, it still contained 0.786 per cent bound gossypol which was more than that in the raw meal. Resistance of bound gossypol to heat is further indicated in the amounts found in sample No. 8 and sample No. 12. Sample No. 8, boiled with steam for 30 minutes, contained 0.761 per cent bound gossypol, and the meats in sample No. 12 which were autoclaved 30 minutes at 10 pounds pressure, contained 0.423 per cent. In the latter case, however, as in the first treatment applied to meal No. 5C, considerable loss of either bound or free gossypol appears to have occurred. The raw meats contained 0.641 per cent free gossypol, but following autoclaving only 0.012 per cent free gossypol and 0.423 per cent bound gossypol were found.

Tests with oil-mill equipment. Data gathered in the laboratory studies were used as a basis for preparation of cottonseed meal by processing cotton seed and expelling the oil, as is regularly done in the oil mills and also by reprocessing commercial cottonseed meal.

In the preparation of meal from cottonseed, delinted seed of the 1942 crop were obtained; the hulls were removed and the meats rolled for cooking. The meats were placed in the cooker and the desired amount of water was added. The cooker agitator was then started and the temperature of the meats raised to the proper level by letting steam into the jacket surrounding the cooker. The meats were cooked until the operator considered them dry enough for pressing, after which

they were placed in a hydraulic press and pressure of 5000 lbs. per square inch applied. When the oil had been expelled, the cakes were removed from the press and ground into meal. The results of these tests are shown in Table 10 in the text.

When cottonseed meats containing 33.4 per cent moisture were cooked for 20 minutes at 270° F. in the pilot cooker, the meal from these meats contained 0.057 per cent free gossypol. This is in contrast with the raw meats, which contained 0.661 per cent free gossypol, but the amount of free gossypol left is as much as that contained in some of the commercial meals. Sample No. 2, heated in the pilot cooker also and prepared from meats containing 48.2 per cent moisture, had only a trace of free gossypol in it. The use of pressure along with heat and moisture appears to have been of little additional value as indicated by a comparison of samples 3 and 4 with samples 1 and 2.

Samples 5, 6, 7 and 8 were prepared in the large cooker at 240° F. All of these were heated with the cooker open, the differences being the moisture contents of the meats and the length of the cooking periods. The free-gossypol content of all the samples prepared in this large cooker ranged downward in approximate proportion to the moisture content of the meats prior to cooking. In general, the reduction of free gossypol in the large cooker at 240° F. was greater than that in the pilot cooker at 270° F. This may be partially explained by the

Table 10

Effect of Moisture and Length of Cooking Period on the Free-Gossypol Content of Cottonseed Meals Prepared by Commercial Processes

Meal sample No.	Type of cooker[a]	Cooking pressure	Cooking temperature	Moisture content	Cooking time	Free gossypol	Pressing condition
		lbs./sq. in.	degrees F.	per cent	minutes	per cent[b]	
1	Pilot	None	270	33.4	20	0.057	Crawled in press considerably
2	Pilot	None	270	48.2	95	0.008	Crawled in press slightly
3	Pilot	10 lbs. 20 min.	270	33.4	30	0.037	Crawled in press excessively
4	Pilot	10 lbs. 20 min.	270	48.2	90	0.018	Crawled in press excessively
5	Commercial	None	240	17.6	50	0.054	Pressed satisfactorily
6	Commercial	None	240	24.5	70	0.038	Pressed satisfactorily
7	Commercial	None	240	35.3	90	0.020	Crawled in press excessively
8	Commercial	None	240	43.3	120	0.007	Crawled in press excessively

[a]Both cookers were of the single-compartment type that could be operated with or without pressure. The pilot cooker was a small experimental cooker which accommodated 25 pounds of meats and formed one press cake from each batch of meats. The commercial cooker approximated normal size and accommodated 300 pounds of meats which formed 12 press cakes.

[b]The raw meats used in preparing samples No. 1, 2, 3, and 4 contained 0.661 per cent free gossypol, and those used in preparing samples No. 5, 6, 7 and 8 contained 0.702 per cent free gossypol. All gossypol percentages are given on a dry-weight basis.

longer cooking period required to drive off the excess water at the lower temperature.

Examination of the column showing pressing condition reveals that only two of the samples pressed satisfactorily, Nos. 5 and 6 containing 17.6 and 24.5 per cent moisture respectively. Sample No. 8, the only sample prepared in the commercial cooker in which the free gossypol was reduced to a trace, was cooked at the highest temperature and for the longest period now employed by most of the mills. Since this treatment resulted in meats that crawled in the press, it is apparent that its use by the mills would necessitate changes in the present milling equipment or procedure. It was noted that some of the meats had a tendency to stick to the agitator and receive less of the cooking effect than the remainder of the material which suggests that improvement of the agitator methods may result in elimination of all the free gossypol by the use of an amount of moisture compatible with good pressing conditions. The results with samples 6 and 7 indicate that sufficient moisture may be used with present mill equipment to produce cottonseed meal containing between 0.020 and 0.038 per cent free gossypol.

The reprocessed meal was prepared from raw commercial cottonseed meal containing 41 per cent protein. The raw meal was placed directly into the cookers and the water added. The agitator was then started and the wet meal raised to the

temperature desired. Cooking was continued until, in the opinion of the operator, the moisture content of the meal was sufficiently low to prevent molding. The meal was then removed from the cooker, spread on the floor and allowed to dry several hours before sacking. The results of these tests are shown in Table 11 which follows:

Table 11

Effect of Moisture, Heat, and Length of Cooking Period on the Free-Gossypol Content of Cottonseed Meal Reprocessed in Oil-Mill Cookers

Meal sample number:	Type of cooker[a]	Cooking temperature	Cooking time	Moisture content	Free-gossypol content[b]
		degrees F.	minutes	per cent	
1	Pilot	240	10	23.8	0.042
2	Pilot	240	15	34.6	0.003
3	Pilot	240	20	49.4	0.003
4	Commercial	240	60	22.0	0.046
5	Commercial	240	79	33.2	0.033
6	Commercial	240	90	39.7	0.012

[a]Refer to foot notes Table 10.

[b]Dry-weight basis. The meal reprocessed to produce samples 1, 2, and 3 contained 0.125 per cent free gossypol before treatment and that used to prepare samples 4, 5, and 6 contained 0.132 per cent free gossypol before treatment.

The raw meal reprocessed in the pilot cooker contained 0.125 per cent free gossypol prior to treatment. Raising the moisture content to 23.8 per cent followed by cooking at 240° F. for 10 minutes, as was done with sample 1, reduced the free-gossypol content to 0.042 per cent. Increasing the moisture content to 34.6 per cent, in the case of sample 2, followed by cooking at the same temperature for 15 minutes eliminated all

but a trace of free gossypol. Samples containing similar moisture levels but cooked in the large cooker contained more free gossypol than those cooked in the pilot cooker even though the cooking period was considerably longer in the large cooker. However, successive addition of moisture and lengthening of the cooking time in the large cooker resulted in further reduction in the amounts of free gossypol in the reprocessed meal. Practically all of the free gossypol was eliminated at 39.7 per cent moisture, as shown by sample No. 6.

IV. DISCUSSION

The data show that both free and bound gossypol exert a toxic effect although the toxicity of the latter is sufficiently mild that control of the free-gossypol content of cottonseed meal is the major consideration in the use of large amounts of this meal for swine. All of the 16 meals tested in these experiments contained sufficient free gossypol to cause death when fed at a level of 25 per cent of the ration. There was wide variation in the free-gossypol content of the several meals, some samples containing four times as much free gossypol as others. On the basis of the swine results the cottonseed meal containing 0.252 per cent free gossypol would prove to be toxic at a level of 9 per cent of the ration which is the amount of cottonseed meal recommended for swine at present.

The studies of factors involved in the milling practices indicate that the mills may manufacture meals that can be safely fed to hogs in larger amounts by varying the practices used in the regular milling process or by reprocessing cottonseed meal.

Detoxicated cottonseed meal for hogs may be produced in the regular oil-milling process by increasing the amount of water used in treating the meats prior to cooking. Although

driving the additional moisture from the meats in the cooking process requires more heat than is commonly used at present, the desired result can be accomplished either by raising the temperature or applying the temperatures now in use over a longer period. At present most of the mills are using stack cookers containing five rings, but are utilizing the full heating effect of only the last two or three rings. By using the full heating capacity of all the rings it appears possible to raise the moisture content of cottonseed meats to between 25 and 30 per cent and cook the moisture out to suitable pressing dryness without changing the mill equipment or slowing the mill operations. The results with sample 6 in Table 10 indicate that sufficient moisture may be used with present mill equipment to produce cottonseed meal containing one-half to two thirds as much free gossypol as the least amount found in any of the commercial meals that were analyzed. Changes in the milling procedure to allow the use of additional moisture and improvement of the agitator methods offer possibilities of further reduction in the free-gossypol content of meals produced. Since the least toxic commercial meal analyzed in this investigation contained an amount of free gossypol that appeared to be near the toxicity threshold for hogs when fed at a level of 25 per cent of the ration, it seems likely that commercial meals may be produced that can be safely fed to hogs in sufficient amount to balance corn or up to 20 per cent of the ration.

The free gossypol contained in cottonseed meal may be eliminated by reprocessing the meal, but further work is necessary before this method can be adopted. More information is needed on the nutritive value of the reprocessed meal, particularly with regard to the effect of the heating process on the biological value of the meal proteins, as well as coordination of the head and moisture factors to avoid scorching or improper drying. Detoxification of meal by this method has certain advantages over that discussed above. It could be used by the mills during the idle seasons which occur at present and therefore would involve very little if any change in the regular oil-extraction procedure. Since cottonseed meal containing a low amount of free gossypol is of importance primarily to swine and chickens, it would be possible to produce special meals for these species without increasing the cost of cottonseed meal for other forms of livestock. The method has an additional advantage in that the bright greenish-yellow color which has been established as desirable in the cottonseed-meal trade would be changed to the brown color characteristic of low free-gossypol meals only in the case of special meals prepared for chickens and swine. It has been estimated that sufficient moisture and cooking time could be applied to cottonseed meal with present mill equipment to eliminate practically all of the free gossypol at an extra cost of one or

two dollars per ton of meal.[1]

Discussion of the two processes has referred to the use of open stack cookers on account of the fact that a majority of the mills are now using this type of equipment. The same principles should be applicable to pressure cookers.

The results obtained from boiling commercial meal and feeding it to hogs are adaptable to practical farm use. By this procedure cottonseed meal may be rendered safe for feeding to hogs in large amounts on the farm through boiling the meal in two and one-half times its weight of water for 30 minutes. In the experiments reported here the meal was prepared twice weekly and fed wet, but the preparation of large amounts of meal followed by proper drying appears feasible.

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[1]Private communication from Mr. John T. Dorsey, Manager, Alabama Oil and Guano Company, Opelika, Alabama, August 28, 1942.

V. CONCLUSIONS

1. The toxicity of cottonseed meal is due to both free and bound gossypol, although the effect of the former is much more severe and the amount of it present in a meal may be used as an index of the toxicity of that meal for hogs.
2. The free-gossypol content of cottonseed meals produced in Alabama varies widely. The lowest in free-gossypol content among the sixteen meals examined in this investigation proved toxic to hogs when fed at a level of 25 per cent of the ration.
3. Application of moisture and heat to cottonseed meats or meal reduced the amount of free gossypol they contain in approximate proportion to the degree of heat, length of time the heat is applied, and moisture content of the meats or meal. The most rapid and complete elimination of free gossypol occurs at temperatures above 100° C. and moisture contents of the meats or meal ranging from 35 to 45 per cent.
4. The effectiveness of present oil-milling procedure in eliminating free gossypol from cottonseed meal may be greatly improved by the use of more moisture in the cooking process. Additional changes in the cooking process may result in meal that can be fed to hogs in sufficient amounts to supply the protein needed for balancing corn or up to 20 per cent of the ration.

5. Cottonseed meal containing little or no free gossypol may be prepared on the farm by boiling the commercial meal thirty minutes in two and one-half times its weight of water. Meal prepared by this method compares favorably with peanut meal as a protein supplement to corn when fed to swine at a level of 25 per cent of the ration.

VI. SUMMARY

Cottonseed meal may be successfully fed to all farm animals. However, with swine and chickens, it is necessary to limit the amounts that are used or feed the meal in combination with an animal source of protein. Under certain conditions it is desirable that larger amounts of cottonseed meal be fed these species. It has been previously determined that the limiting factor is a toxic substance, gossypol, which occurs in cottonseed meal in both a "free" and a "bound" form, the proportions of which are influenced by heat and moisture. It was the purpose of this investigation to study the occurrence of free and bound gossypol in commercial cottonseed meal; their relationships to the toxicity of the meal; and the possible reduction or elimination of the toxicity to an extent which would allow the use of more cottonseed meal as a protein supplement to corn in the rations of swine.

Meals from sixteen mills in Alabama were obtained and analyzed for their contents of free and bound gossypol. The free-gossypol contents ranged from 0.055 to 0.252 per cent and the bound-gossypol contents from 0.627 to 1.041 per cent. Experiments with rats and chicks showed that the toxicity of these meals was due primarily to the free gossypol they contained and that this substance could be rendered non-toxic by

treatment with moisture and heat. Similar results were obtained with swine. The meal containing the least amount of free gossypol proved toxic to hogs when fed at a level of 25 per cent of the ration, while the meal which contained the largest amount of free gossypol and which killed six out of eight hogs when fed in the commercial form, proved non-toxic after the free gossypol was greatly reduced or eliminated.

Oil-mill and laboratory studies showed that the application of moisture and heat to cottonseed meats or meal reduces the amount of free gossypol they contained in approximate proportion to the degree of heat, length of time the heat is applied, and moisture content of the meats or meal. The most rapid elimination of free gossypol occurred at temperatures above 100° C. and moisture contents of the meats or meal ranging from 35 to 45 per cent. Free gossypol was practically eliminated from cottonseed meats and cottonseed meal by treatment at these levels of heat and moisture. Apparently the greatest improvement that may be made in present oil-milling procedure to further eliminate free gossypol from the cottonseed meals produced is the use of more moisture in the cooking process. The data indicate that the use of maximum temperatures and cooking time employed by the mills at present, with the addition of as much moisture as is compatible with satisfactory pressing conditions, will result in cottonseed meal containing approximately one-half the least amount of free gossypol found in any of the commercial meals analyzed. Additional changes in the

cooking process may result in meal that can be fed to hogs in sufficient amounts to supply the protein needed for balancing corn up to 20 per cent of the ration. Free gossypol was practically eliminated from commercial cottonseed meal by raising the moisture content of the meal to between 35 and 45 per cent and reprocessing it in oil-mill cookers. This method should receive further study before being adopted commercially.

Cottonseed meal containing little or no free gossypol was prepared by boiling commercial meal thirty minutes in approximately two and one-half times its weight of water and leaving it in the container until cool. Meal treated in this manner was considerably improved for chicks and compared favorably with peanut meal as a protein supplement to corn for hogs at a level of 25 per cent of the ration.

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APPENDIX

Table 1

Weights and Feed Consumption of Rats Fed Rations Supplemented with Sixteen Cottonseed Meals Produced in Alabama

Meal number	Group feed consumption	Kind of weight	Rat ear mark and weight[a] O	L	R	C
	gms.		gms.	gms.	gms.	gms.
Basal peanut meal	3,501	Initial	99	91	83	85
		Final	246	230	242	264
1	3,584	Initial	94	81	103	81
		Final	200	159	214	162
2	2,903	Initial	86	100	86	86
		Final	118	200	206	172
3	2,841	Initial	101	80	95	85
		Final	192	143	170	138
4	3,202	Initial	101	74	92	93
		Final	136	110	146	113
5	2,046	Initial	89	94	94	86
		Final	161	133	167	103
6	3,175	Initial	101	91	85	85
		Final	179	160	188	220
7	2,950	Initial	88	95	84	93
		Final	192	216	163	183
8	3,096	Initial	103	81	85	92
		Final	252	100	176	172
9	3,154	Initial	83	85	100	88
		Final	170	169	220	186
10	3,311	Initial	93	88	84	91
		Final	214	190	170	176
11	2,898	Initial	90	91	92	84
		Final	146	156	148	177
12	3,242	Initial	84	86	87	101
		Final	178	192	218	208
13	3,236	Initial	99	80	86	99
		Final	200	158	170	242
14	1,301	Initial	90	82	98	85
		Final	90	(19)[b]	118	(16)[b]
15	3,442	Initial	98	76	90	99
		Final	246	145	204	210
16	3,887	Initial	97	101	85	77
		Final	230	241	204	172

[a]Feeding period of 9 weeks.

[b]Parentheses indicate rats that died. The numbers enclosed represent the number of days the rats survived.

Table 2

Average Gains by Weeks of Rats Fed Rations Supplemented with Peanut Meal and with Cottonseed Meals Containing Different Amounts of Free Gossypol

Supplement	Basis of calculated gains	Free gossypol in meals	Average gain per rat above average initial weight by weeks[a]								
			1	2	3	4	5	6	7	8	9
		per cent	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
Peanut meal	Group mean	check	23	46	61	82	111	132	139	153	156
Least toxic cottonseed meal	Group mean	0.056	24	41	56	82	101	119	116	116	122
Most toxic cottonseed meal	Group mean	0.164	-2	-7	-7	0	6	15	12	9	10
Seven cottonseed meals	Average of group means	0.055 to 0.075	22	38	53	68	81	96	98	97	100
Three cottonseed meals	Average of group means	0.075 to 0.095	27	42	54	71	86	97	100	96	99
Two cottonseed meals	Average of group means	0.095 to 0.115	19	34	43	57	71	82	86	88	85
Two cottonseed meals	Average of group means	0.115 to 0.135	17	26	32	43	52	56	57	56	53
Two cottonseed meals	Average of group means	0.155 to 0.175	4	11	12	16	25	36	31	31	30

[a]The gains of rats shown for single meals were calculated by subtracting the mean initial weight per rat for each group from the mean weight per rat at the end of each weekly period. Combinations of two or more meals represent averages of the several group means. All of the groups contained four rats at the beginning of the experiment. One rat receiving the most toxic meal died in the third week and another in the fourth week of the experiment. This affected the gains of both the individual meal and the combination in which it was included.

Table 3

Weights of Chicks Fed Rations Supplemented with Cottonseed Meals Containing Different Kinds and Amounts of Gossypol

Supplement	Cottonseed meal 16C		Cottonseed meal 14C		Cottonseed meal 5C	
	Initial weight	Final weight[a]	Initial weight	Final weight[a]	Initial weight	Final weight[a]
Chick no.	gms.	gms.	gms.	gms.	gms.	gms.
1	42	220	36	240	40	(9)[b]
2	38	200	38	100	38	120
3	40	(3)[b]	35	180	44	(25)
4	38	200	38	(33)[b]	32	(13)
5	42	(6)	38	200	40	130
6	40	160	40	140	44	120
7	38	(9)	38	(31)	38	120
8	40	(6)	38	160	38	(24)
9	40	(5)	38	140	38	80
10	38	200	38	180	38	(31)
11	40	300	32	220	38	(22)
12	42	300	40	200	38	164
13	38	200	32	200	32	100
14	38	200	42	200	38	(21)
15	40	(10)	32	160	38	110
16	42	280	40	(6)	40	(10)
17	40	240	38	140	38	100
18	38	(20)	40	220	38	120
19	32	220	40	(6)	44	140
20	40	(26)	42	440	38	88
21	32	140	38	200	38	(16)
22	58	(8)	40	340	40	120
23	40	200	40	180	44	(19)
24	40	290	30	100	40	(27)
25	42	240	44	(10)	40	(23)
26	36	220	40	240	38	(12)
27	38	260	42	300	38	(1)
28	40	240	38	(36)	40	(32)
29	38	200	36	200	40	(15)
30	40	180	34	160	40	140
31	30	200	38	260	38	(17)
32	40	(15)	38	200	46	(27)
33	38	240	40	(9)	38	(16)

[a]Feeding period of 6 weeks.

[b]Parentheses indicate the chick died before the end of the experiment and the number enclosed represents the number of days the chick survived.

Table 4

Weights of Rats Fed Rations Supplemented with Peanut Meal, Raw Cottonseed Meals and Cottonseed Meal Treated with Moisture and Heat

Series	Supplement	Kind of weight	Rat ear mark and weight[a] O	L	R	C
			gms.	gms.	gms.	gms.
I	Peanut meal	Initial	79	79	86	66
		Final	240	226	237	231
	Cottonseed meal No. 16, raw	Initial	84	79	81	67
		Final	211	200	181	188
	Cottonseed meal No. 14, raw	Initial	84	80	75	74
		Final	87	94	(24)[b]	102
	Cottonseed meal No. 14, treated[c]	Initial	81	83	69	80
		Final	194	176	192	187
	Cottonseed meal No. 14, treated[d]	Initial	70	80	78	84
		Final	198	179	188	200
II	Peanut meal	Initial	69	70	63	65
		Final	270	251	226	216
	Cottonseed meal No. 16, raw	Initial	70	62	70	63
		Final	201	178	204	232
	Cottonseed meal No. 5C, raw	Initial	65	73	66	64
		Final	(54)	(51)	76	(46)
	Cottonseed meal No. 5C, treated[d]	Initial	70	63	64	69
		Final	170	194	180	220

[a]Feeding period of 8 weeks.

[b]Parentheses indicate rats that died. The numbers enclosed represent the number of days the rats survived.

[c]This meal was mixed with one and one-half times its weight of cold water and dried at 70° C.

[d]These meals were mixed with one and one-half times their weights of boiling water and dried at 70° C.

Table 5

Mean Gains by Weeks of Rats Fed Rations Supplemented with Peanut Meal, Raw Cottonseed Meal, and Heat-Treated Cottonseed Meal

Supplement	Mean gain per rat above mean initial weight, by weeks[a]							
	1	2	3	4	5	6	7	8
	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
Peanut meal	28	60	68	87	114	126	139	156
Cottonseed meal No. 14, raw	7	-9	-0.5	4	13	8	15	11
Cottonseed meal No. 14, heat treated	23	45	53	70	89	97	112	113

[a]The gains shown were calculated by subtracting the mean initial weight per rat for each group from the mean weight per rat at the end of each weekly period. Each group contained four rats except the group supplied raw meal in which one rat died during the fifth week leaving three rats in this group for the remainder of the experiment.

Table 6

Weights of Chicks Fed Rations Containing Supplements of Animal-Vegetable Origin, Raw Cottonseed Meal and Cottonseed Meal Treated with Moisture and Heat

Supplement	Animal and vegetable protein balanced		Cottonseed meal 5C, raw		Cottonseed meal 5C, heat treated	
	Initial weight	Final weight[a]	Initial weight	Final weight[a]	Initial weight	Final weight[a]
Chick no.	gms.	gms.	gms.	gms.	gms.	gms.
1	35	400	36	120	36	436
2	35	422	40	372	38	(38)[b]
3	32	440	28	(38)[b]	36	260
4	35	332	36	188	36	200
5	35	256	36	268	35	220
6	35	420	35	140	40	260
7	37	444	37	(24)	36	248
8	37	324	36	120	36	288
9	35	420	38	232	36	280
10	30	396	40	180	40	256
11	35	300	36	(34)	38	256
12	35	440	36	164	34	224
13	30	428	37	180	36	224
14	30	444	36	200	36	260
15	38	252	36	(39)	38	(5)
16	30	420	36	204	36	332
17	36	428	36	228	36	252
18	30	264	36	116	36	328
19	31	360	36	(2)	34	228
20	30	360	37	(32)	36	228
21	31	356	37	(19)	36	228
22	40	380	34	180	35	280
23	38	400	35	132	38	240
24	36	516	36	264	34	220
25	36	400	35	280	36	320
26	32	420	34	112	36	140
27	36	500	37	184	32	244
28	35	328	36	208	32	148
29	36	408	36	220	36	284
30	39	440	34	140	34	312
31	32	396	36	232	34	(5)
32	39	368	38	204	36	320
33	32	260	36	(40)	32	280

[a]Feeding period of 6 weeks.

[b]Parentheses indicate the chick died before the end of the experiment, and the number enclosed represents the number of days the chick survived.

Table 7

Feed Consumption and Weights of Hogs Fed Rations Su
Cottonseed Meals Containing Different Kinds a
and Cottonseed Meal Treated with Moi

Lot no. and supplement	Lot feed consumption	Duration of exp.			Pig
	lbs.	wks.			
Lot I Peanut meal	5,845.90	20	Pig no. Initial wt. Final wt.	51 30.0 164.67	58 30. 179.
Lot II Cottonseed meal No. 16C	5,190.90	20	Pig no. Initial wt. Final wt.	17 28.33 202.00	4 30. 181.
Lot III Cottonseed meal No. 4C	4,241.70[b]	20	Pig no. Initial wt. Final wt.	6 30.00 150.33	25 26. 160.
Lot IV Cottonseed meal No. 5C	2,286.30[b]	20	Pig no. Initial wt. Final wt.	30 36.67 213.33	5 41. 63. (5
Lot V Cottonseed meal No. 5C cooked[c]	5,899.30[b]	20	Pig no. Initial wt. Final wt.	28.00 43.33 228.00	31 33. 183.
Lot VI Cottonseed meal No. 5C cooked[c]	5,480.50	18	Pig no. Initial wt. Final wt.	22 36.67 165.00	6 43. 182.

[a]Weights shown are the means of weighings made on three successive day
indicate the animal died before the experiment closed and the figure encl
The weight shown is that for the last weighing period before death.

[b]Feed consumption for lots in which deaths occurred was corrected for
fore the death happened by subtracting the average amount of feed consume

[c]For method of treatment see footnotes of Table 6 in the text.

Table 7

Hogs Fed Rations Supplemented with Peanut Meal,
ng Different Kinds and Amounts of Gossypol
eal Treated with Moisture and Heat

	Pig number and weights[a]							
no.	51	58	26	16	2.00	70	14	38
tial wt.	30.0	30.00	36.67	28.33	40.00	30.00	41.67	35.00
al wt.	164.67	179.67	208.00	192.33	240.67	182.00	235.33	212.67
no.	17	4	36	50	13	9	39	28
tial wt.	28.33	30.00	38.33	36.67	31.67	33.33	31.67	33.33
al wt.	202.00	181.33	223.00	95.00 (11)	155.00	165.67	151.33	203.00
no.	6	25	117	27	44	22	48	21
tial wt.	30.00	26.67	40.00	40.00	43.33	28.33	33.33	30.00
al wt.	150.33	160.33	182.67	82.00 (7)	148.00 (17)	154.67	175.00	177.00
no.	30	5	34	21	23	15	20	35
tial wt.	36.67	41.67	26.67	30.00	41.67	40.00	30.00	28.33
al wt.	213.33	63.00 (5)	47.00 (5)	55.00 (9)	110.00 (14)	237.00	60.00 (6)	60.00 (11)
no.	28.00	31	3	7	11	42	26	12
tial wt.	43.33	33.33	41.67	33.33	30.00	28.33	31.67	31.67
al wt.	228.00	183.33	217.00	250.67	197.00	210.33	180.00	225.00
no.	22	6	23	43	41	47	60	68
tial wt.	36.67	43.33	41.67	50.00	45.00	48.33	41.67	41.67
al wt.	165.00	182.00	232.67	245.67	205.33	243.00	228.33	200.33

three successive days at the start and close of the experiment. Parentheses
and the figure enclosed denotes the number of weeks the animal survived.
before death.

ed was corrected for the pigs that died back to the last weighing period be-
nount of feed consumed per pig.

in the text.